Large-scale restoration of eelgrass (*Zostera marina*) in the Potomac River, Maryland

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Project Information

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Executive Summary:

This report summarizes the first two years of a three-year project to restore eelgrass to the lower Potomac River. In 2003 and 2004, approximately 3,000,000 eelgrass seeds were sown in four areas using spring seed bags and fall seeding. These two methods are compared to nearby adult test plantings. Eelgrass seeding and subsequent survival are summarized as follows:

Site	Seeding Method	Season/ Year of seeding	Plot Size (Acres)	Initial recruitment	Plants survived through fall 2005
Piney Point	Seed bags	fall/2003	3	No	No
Piney Point	Seed broadcast	Fall/2004	0.5	No	No
St. George Island	Seed bags	Spring/2004	5	Yes	Yes
St. George Island	Seed broadcast	Fall/2004	0.25	Yes	Yes
Sage Point	Seed bags	Spring/2004	10	Yes	No
Cherryfield Point	Seed bags	Spring/2004	5	Yes	No
Cherryfield Point	Seed broadcast	Fall/2004	0.25	No	No

The water quality data collected show prolonged periods of poor water clarity while water temperatures are above 25 degrees. The combination of high light attenuation by epiphytes, low ambient light levels, and high temperatures in the summer of 2004 and 2005 are probably responsible for the loss of most of the seedlings and adult plants. Despite 2003, 2004, and 2005 being exceptionally poor years for water clarity, including setting new 20-year record lows for monthly Secchi depth on 8 separate occasions during the three year period, 2 of the 7 method/year combinations had plants at the time of our last survey.

Evaluation of the cost effectiveness of the three methods used showed that spring seed bags were by far the most cost effective restoration technique, with a cost per seedling of $1.70 compared to $4.70 for planting an adult plant and $363.89 per seedling for fall seed broadcasting. However, the extremely high fall seed broadcast cost estimate is the result of seed loss during summer storage, a condition that improved storage conditions may offset in 2006. Method comparisons will be performed again in 2006.

Introduction:

Submerged Aquatic Vegetation Declines in the Chesapeake Bay

Worldwide, eelgrass (*Zostera marina*) abundance has declined significantly since the turn of the century due to pollution associated with increased human populations (Short and Wylie Echeverria, 1996) and episodic occurrences of the 'wasting disease' (Short et al, 1986; den Hartog, 1994). In Chesapeake Bay, deforestation, population growth, and the subsequent sedimentation and nutrient enrichment caused declines in all species of submerged aquatic vegetation (SAV) beginning in the 17th century (Brush and Davis, 1984). However, those changes were relatively minor compared to the catastrophic declines that occurred in the late 1960's and early 1970's (Orth and Moore, 1983).

Researchers suggest a combination of factors resulted in these losses. Kemp et al. (1983) and Twilley et al. (1985) postulated that increased nutrient loadings of the Chesapeake Bay in the 1970's enhanced growth of planktonic and periphytic algal species which compete with SAV for light. SAV productivity was shown to be reduced further when suspended sediment increases in the water column, exacerbating light attenuation problems (Wetzel and Penhale, 1983; Kemp et al., 1983). These studies demonstrated that SAV growth and abundance were inextricably linked to water quality. In addition to the combined effects of degraded water quality, the flooding that accompanied Hurricane Agnes in 1972 resulted in a prolonged period of high suspended sediment loads at a critical time of year for SAV growth. The combination of stressors had devastating effects on the SAV acreage baywide, and few areas have recovered to their 1930's- 1950's levels.

SAV Restoration in the Chesapeake Bay

SAV is widely recognized as an aquatic habitat vital to the health of Chesapeake Bay, and its restoration has long been a goal of the U.S. Environmental Protection Agency's Chesapeake Bay Program (CBP) and its partners. The CBP has recently completed a "Strategy to Accelerate the Protection and Restoration of Submerged Aquatic Vegetation in the Chesapeake Bay". One of the goals of this strategy is to plant or reseed 1,000 acres in strategic locations by December 2008.

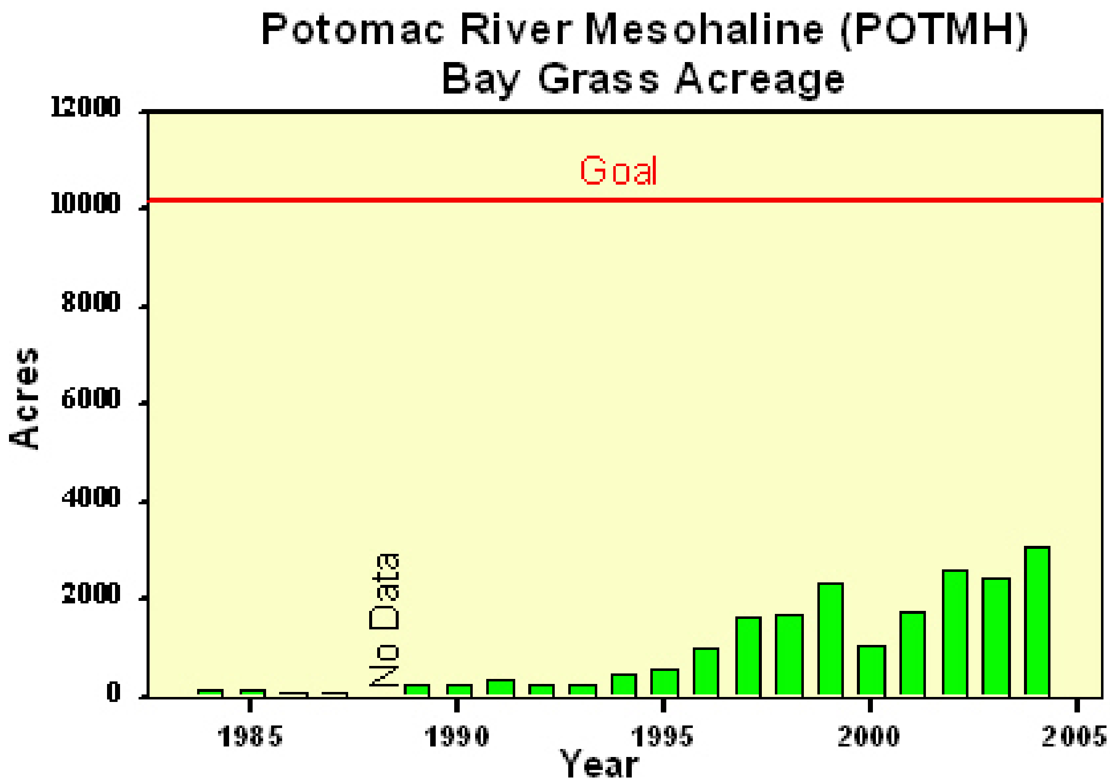


Figure 1. SAV acreage in the mesohaline portion of the Potomac River.

SAV acreage in Chesapeake Bay was estimated to be approximately 72,935 acres in 2004, less than 40% of the CBP's 185,000 acre SAV goal. Even the auspicious goal of planting 1,000 acres by 2008 will provide only small progress toward the baywide goal. However, it is recognized that there are regions within the Chesapeake Bay in which habitat conditions are suitable for SAV growth, but SAV has not recovered due to a lack of adequate seed or propagule sources. By identifying and strategically planting or reseeding beds in these areas, it is expected that these beds would serve as a seed source to accelerate natural revegetation.

<u>Eelgrass in Restoration</u>

Eelgrass is identified in the CBP's SAV Protection and Restoration Strategy as one of the two species with the greatest promise for large-scale restoration in Chesapeake Bay. Eelgrass meadows are highly productive components of estuarine and coastal systems and support large and diverse faunal assemblages (Thayer et al., 1984). Eelgrass plants filter and absorb nutrients from the water column (Short and Short, 1984), provide sediment stabilization (Ward et al., 1984) and baffle wave energy (Fonseca and Fisher, 1984) thereby reducing erosional forces and protecting adjacent shorelines (Christiansen et al., 1981). Eelgrass biomass production serves as a major component of the detrital food chain (Thayer et al., 1984).

As eelgrass is locally extinct in several areas of Chesapeake Bay, eelgrass restoration has been ongoing in Chesapeake Bay since 1980. Experiments have been performed with three different restoration techniques : 1) shoots with sediments intact, (2) seeds, and (3), shoots with bare roots. This study compared the cost effectiveness of using of seeds or adult plants for restoration in the Potomac River.

In the past, there have been a wide variety of small-scale (less than 1 acre) SAV planting, transplanting, and reseeding efforts in Chesapeake Bay that have generally met with poor success. The consensus among SAV researchers is that this is due primarily to two factors. First, bed size has been too small to afford self protection. Water clarity improves significantly within a few meters of the edge of the bed. Large beds are capable of self-protecting the core of the bed, while allowing the fringes to die back during periods of poor water clarity. Large beds are also better able to withstand damage by predators, such as mute swans (*Cygnus olor)* and cownose rays (*Rhinoptera bonasus*). Second, many previous projects have suffered from improper siting. Restoration sites have typically been selected for convenience and practicality rather than where habitat assessments and test plantings have indicated suitable areas where SAV restoration is likely to be successful.

To address these issues, the SAV Protection and Restoration Strategy calls for large-scale projects that are implemented over the course of five years. The first two years of the project are devoted to site selection, which involves applying existing habitat information to identify general areas suitable for restoration followed by test plantings at specific sites. Once the sites are selected, large-scale planting or reseeding is spread over a three-year period to minimize impacts from adverse environmental conditions in any single year.

Submerged Aquatic Vegetation Restoration in the Potomac River

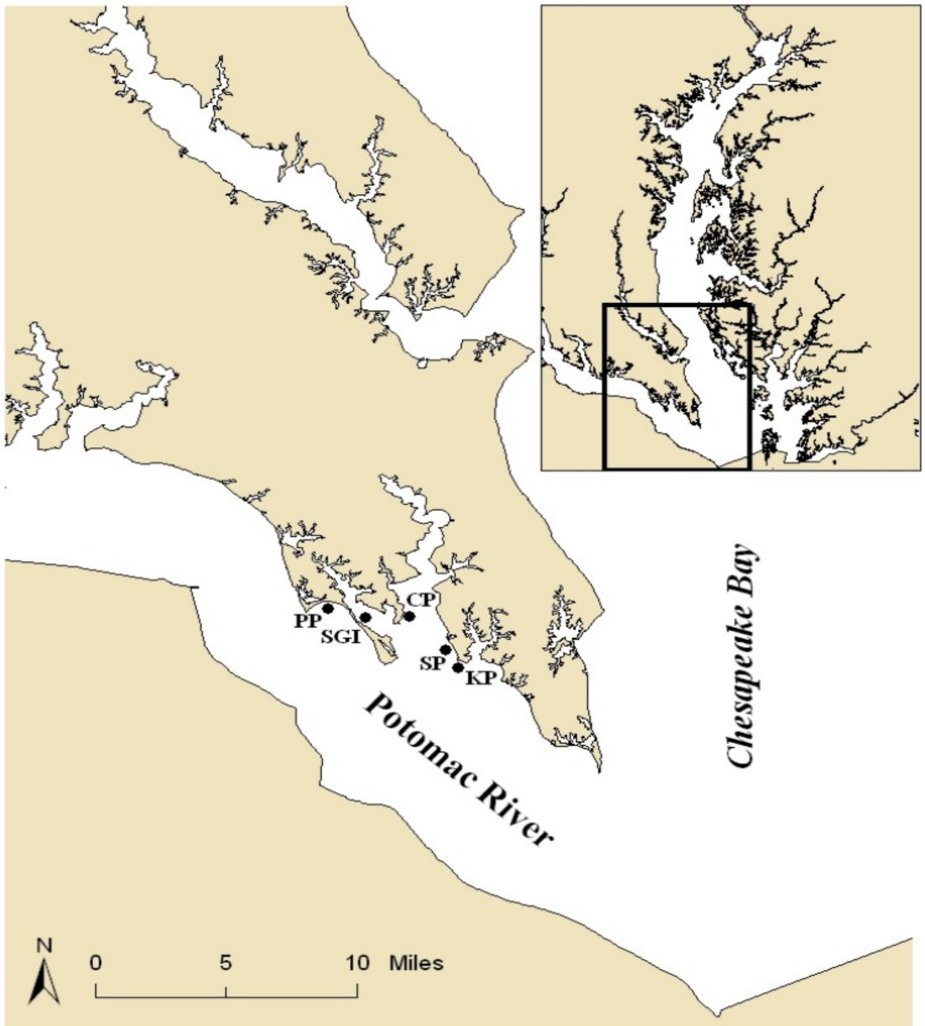


Figure 2. Map of Potomac River with restoration sites (inset, map of Chesapeake Bay). Cherryfield Point (CP), Piney Point (PP), Sage Point (SP), St. George Island (SGI), and Kitt's Point (KP).

The Potomac River is the largest river in Maryland, with a drainage area of 14,679 square miles in four states. The majority of the Potomac basin's land area is covered by forests (57.6%), followed by agriculture (31.8%), water and wetlands (5%) and developed land (4.8%, Potomac River Basin Summary, DNR website). As a large tidal river, the Potomac has significant freshwater (0 ppt salinity), oligohaline (0.5-5 ppt), and mesohaline (5-18 ppt) reaches, each with their own unique water quality attributes. SAV coverage in the tidal fresh portion has increased from a low of 1,134 acres in 1984 to 2,410 acres in 2004, approximately 55% of the 4,368 acre CBP goal. The oligohaline section during the same time period increased from 429 acres to 3,734 acres, exceeding the 3,721 acre goal in that reach of the river. Although the mesohaline section of the river increased from 109 acres in 1984 to 3,401 acres in 2004 (Fig. 1), this is only 33% of the 10,173 acre goal, the lowest attainment by far of the three segments (Orth et. al 1985, Orth et. al 2005). This is likely due to the fact that eelgrass, once a dominant species in the area, has not been documented in the Potomac River for decades.

The Maryland Department of Natural Resources (DNR) has developed a five year plan to conduct large-scale eelgrass restoration on the Potomac River. This plan represents a synthesis of restoration work already conducted and a variety of new technologies that will maximize the acreage that can be restored. The effort will focus primarily on the use of seeds for restoration, and will compare the effectiveness of two different broadcast methods to determine the most efficient and productive way to achieve the bay wide restoration goal of 1,000 acres by 2008. At the same time, DNR will compare the effectiveness of the seeding with large-scale restoration using vegetative shoots in a side by side comparison. The project will also test the effectiveness of depositing seeds in different seasons using several techniques. This will allow for a direct comparison between seeds vs. adult shoots, as well as the effectiveness using spring seed bags vs. fall seed dispersal.

Research Methods:

Site selection

Locations for large-scale restoration activity were determined using a Geographic Information System (GIS) based SAV restoration targeting model (Parham and Karrh 1998). The model uses six layers of key habitat information to evaluate the suitability, ability and potential of a particular habitat to support SAV populations. The data layers incorporated into the targeting model include:

1. Shoreline: The Maryland shoreline datalayer used was digitized by the Soil Conservation District using United States Geological Survey (USGS) quad sheets at a scale of 1 inch = 24,000 feet.
2. Water Quality: The water quality parameter allows site evaluation based on three methods: Percent light at leaf, percent light at water (Kemp et al., 1995), or the individual water quality parameters (Dennison, 1993). Six water quality parameters important to SAV communities were incorporated into the SAV Restoration Targeting System (light extinction coefficient (Kd), dissolved inorganic nitrogen (DIN), dissolved inorganic phosphorous (DIP), total suspended solids (TSS), chlorophyll a (Chla) and salinity). Data from a running three year growing season (April to October) for SAV were used to obtain a median value by station for each parameter. The data were obtained from the Chesapeake Bay Mainstem

and Tributary Water Quality Monitoring Program, intensive surveys, and water quality mapping. The individual water quality parameters were interpolated using the Inverse Distance Weighted interpolation method in Spatial Analyst for ArcView using four nearest neighbors and 100 foot interpolated cells extending beyond the extent of the Chesapeake Bay. After interpolation of the individual parameters, each parameter was overlayed with salinity coverage and assigned as pass or fail based on the SAV habitat requirements for one meter restoration (Batuik et al., 1992).

3. Bathymetry: One and two-meter bathymetry contours for the Chesapeake Bay were obtained from the Environmental Protection Agency's, Chesapeake Bay Program, intersected with the Soil Conservation District shoreline and converted from lines to polygons. The resulting shapes were designated to yield areas less than 1 meter depth at mean low water, areas 1 to 2 meters depth and areas greater than 2 meters depth.
4. Submerged Aquatic Vegetation: SAV distribution coverage data was determined based on aerial surveys completed by the Virginia Institute of Marine Science (1981-2004). Current distribution was composed of the 2003-2004 SAV distribution. A composite layer of historical SAV distribution was created by combining the 1981, 1984-1990, and 1991-2004 SAV aerial surveys.
5. Hydraulic Clam Dredging: Prohibited clamming areas were mapped based on the laws in the Code of Maryland regulating this activity (§4-1037 and §4-1038). DNR natural oyster bar habitats were buffered by 150 feet as called for in the State and County laws, and a shoreline setback was established and buffered to the appropriate distance (distance varying by County) using the Soil Conservation District Shoreline coverage.

Study area

Five sites in the lower Potomac River were identified as suitable for eelgrass recolonization based on the DNR SAV targeting model (Fig. 3).

- Cherryfield Point (N38° 07.819′ W76° 27.574′)
- Piney Point (N38° 08.279′ W76° 30.159′)
- Sage Point (N38° 07 53.2′ W076° 26 10.5′)
- St. George Island (N38° 08 07.6′ W076° 29 41.4′)
- Kitt's Point (N36° 06.628′ W76° 25.471′)

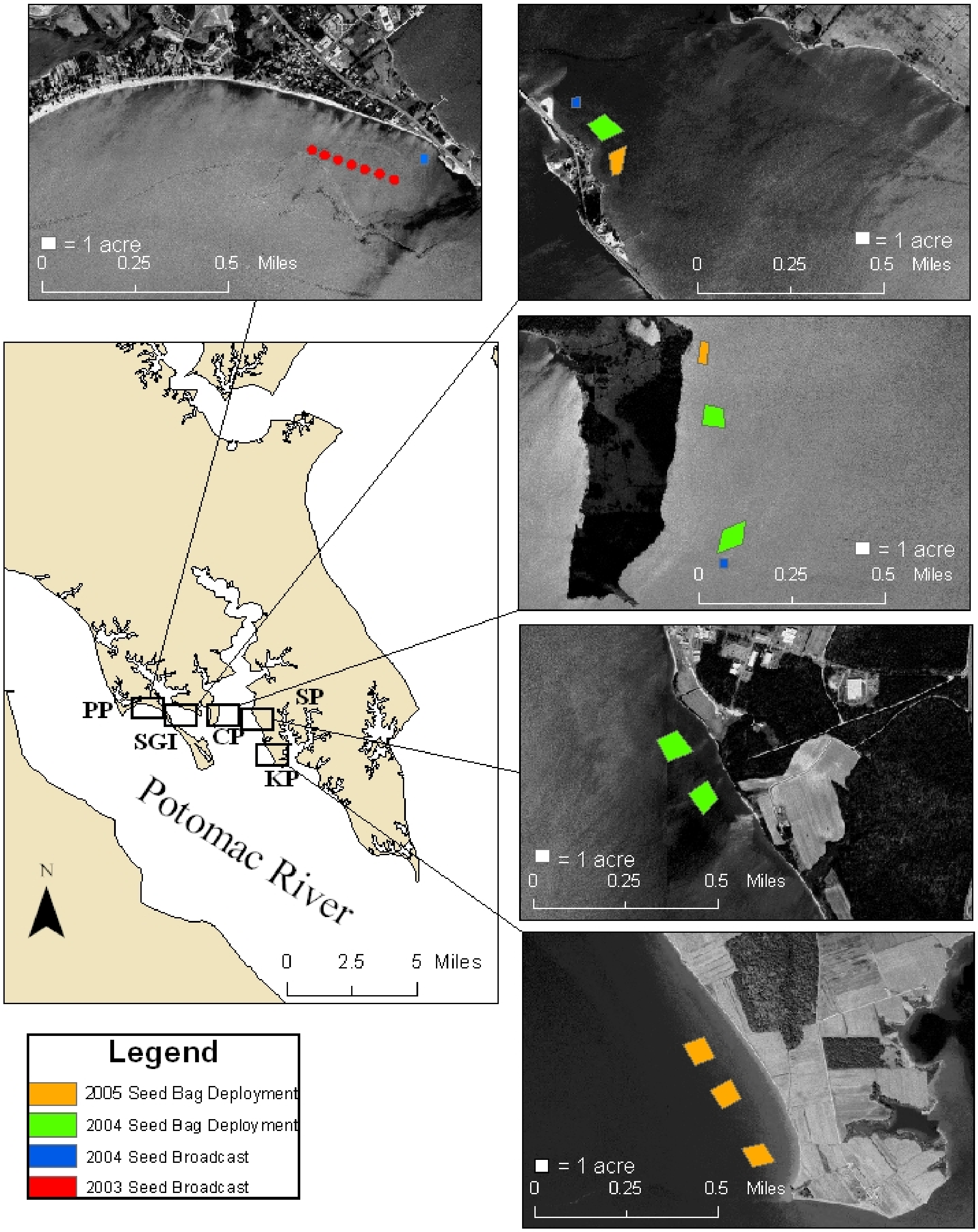


Figure 3. Study area with restoration sites. Cherryfield Point (CP), Piney Point (PP), Sage Point (SP), St. George Island (SGI), and Kitt's Point (KP).

Test plantings

To determine the best planting sites within the areas identified by the SAV restoration targeting model, adults plants raised in the laboratory and harvested from existing beds in the bay were transplanted into three, one square meter plots in areas adjacent to seed broadcast and seed bag areas. Sixty-four adult plants were planted in each plot, anchored by wooden skewers (Davis, 1997). These test plantings were monitored for percent survival at 1 week, 4 weeks and 16 weeks after initial planting.

Adult shoot plantings

As part of the Compensatory Mitigation Package for the Woodrow Wilson Bridge, 20 acres of SAV are being planting by Rummel, Klepper and Kahl (RK&K). The first year's planting of this three year project was 3.5 acres planted in the fall of 2003 with 16,816 planting units (PUs) of widgeon grass (*Ruppia maritima*) and 2,016 PUs of sago pondweed at Sage Point, and 2 acres planted with 15,000 PUs of eelgrass, 964 PUs of sago pondweed and 1,600 PUs of widgeon grass at Piney Point. In spring of 2004, 39,456 sago pondweed PUs were planted at Piney Point. In the fall of 2004 an additional 19,440 PUs of eelgrass, 7,488 PUs of sago pondweed, and 21,479 PUs of widgeon grass were planted at Piney Point, and 2,016 PUs of sago pondweed and 16,816 PUs of widgeon grass were planted at Sage Point.

Seed Collection

To begin the project, DNR staff concentrated efforts on finding the most productive donor beds from which to harvest. Because the importance of temperature on the life cycle of eelgrass, especially on reproduction, latitudinal comparisons should show a progression of stages in the reproductive cycle (anthesis and seed release) as one moves south (Silberhorn, 1983). In the Chesapeake Bay's eelgrass beds, anthesis (the period during which a flower is fully open and functional) was observed when temperatures were nearly 15^0 C, and above 20^0 C, flowers and immature fruits die and slough off the plant (Silberhorn, 1983).

Figure 4. The mechanical harvester collects eelgrass reproductive shoots.

Since the Chesapeake Bay is close to the southernmost reach of eelgrass distribution, eelgrass flowering and seed release begins in April and May. In 2003, eelgrass reproductive shoots were collected manually from donor beds in Sinepuxent Bay and Tangier Sound. Seed broadcast techniques vary in success with around 15% of viable seeds becoming established (Orth et al., personal communication; Orth et al., 1994), so it is necessary to harvest large numbers of seeds to achieve restoration potential. For approximately 3 weeks, DNR staff and volunteers snorkeled and used scuba equipment to manually remove the reproductive shoots of eelgrass.

This was a very expensive process in terms of man-hours involved, so over the winter, alternative methods of harvesting were investigated. In the past, DNR has contracted the use of a mechanical harvesting boat used for clearing boating channels to harvest water chestnut (*Trapa natans*). It was found that very little work had to be done to adapt this harvester to collect eelgrass reproductive shoots. The reproductive shoots stand above a majority of the plant biomass and could be harvested with little or no impact on the eelgrass beds. During subsequent harvests (2004 and 2005), a mechanical harvest boat was utilized (M J McCook & Associates, La Plata MD) to increase the volume of reproductive material collected.

Historically, Tangier Sound and the Little Annemessex River have healthy eelgrass beds and in 2004 and 2005 served as donor beds. During aerial surveys of these areas, the areas with the highest density of plants were identified as areas to focus the harvesting efforts. DNR visited each of these donor beds weekly beginning the second week in April. Reproductive structures begin to form when water temperatures reach 10^{o} - 15^{o} C (Granger, 2002). Flowering is completed and viable seeds begin to develop when water temperatures reach 15^{o} -20^{o} C (Granger, 2002). Random samples of reproductive shoots were collected and analyzed to determine the maturity of the seeds.

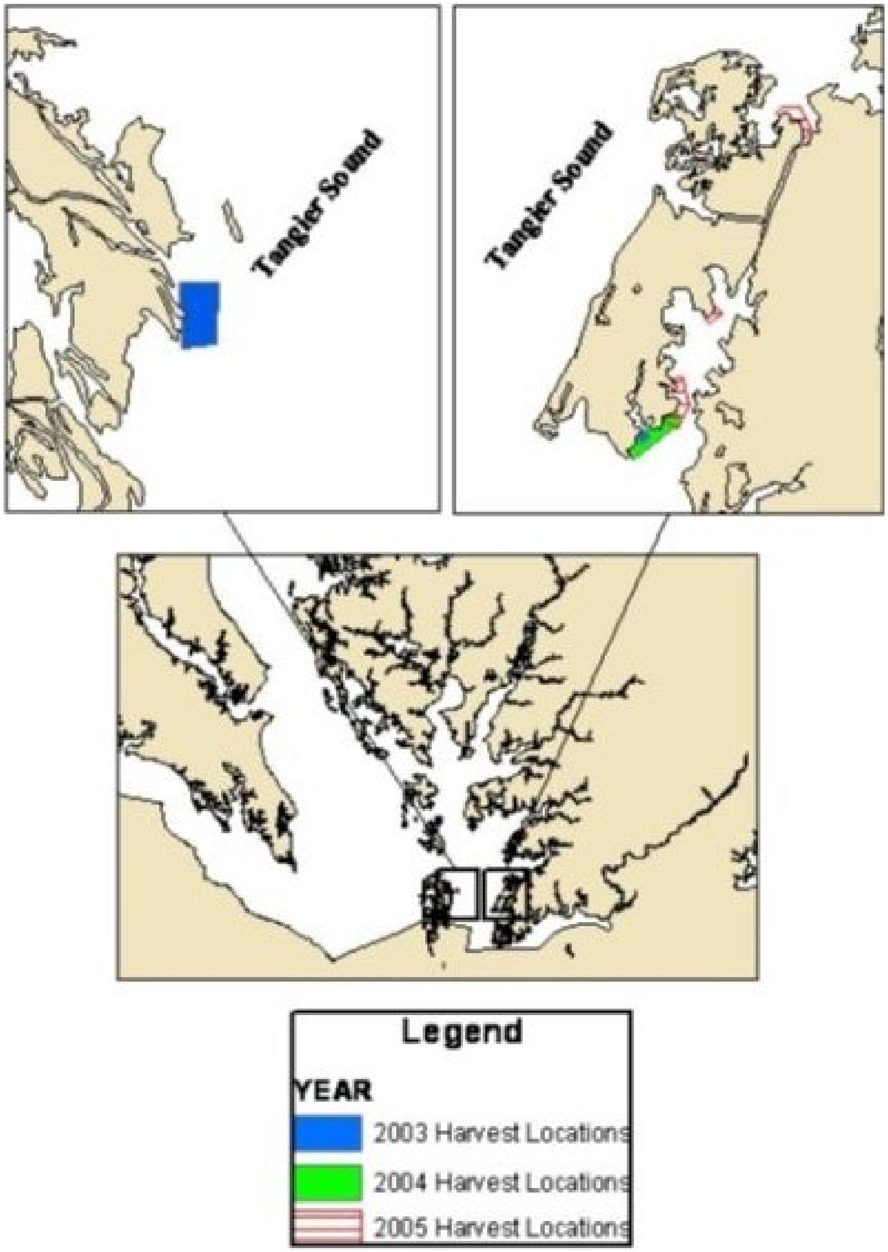


Figure 5. Harvest sites for 2003-2005, Tangier Sound, MD.

When more than 50% of the seeds were mature, and the spathes had begun to drop the seeds, DNR mobilized its field staff to the Tangier Sound eelgrass beds to begin harvesting.

Seeds were collected from donor beds in the Little Annemessex River and Tangier Sound near Smith Island (N37° 58.479′ W75° 52.255′ and N37° 59.073′ W75° 59.206′, respectively) and in 2005 from the Little Annemessex River and the mouth of Acre Creek (Big Annemessex River (N37° 59.626′ W75° 51.636′ and N38° 01.718′ W75° 50.632′, respectively). The harvester would run systematic transects within the beds, adjusting the cutting blades to account for changes in depth. As the boat moved slowly through the water, the cutting blades clipped the eelgrass reproductive shoots at approximately one foot above the sediment (Fig. 5). The cuttings were sent to the conveyor belt and stored in

Figure 6. Harvesting boat unloading eelgrass.

the hoppers on the back of the boat. Once the boats hoppers were full, a DNR boat would dock with the harvester, and the harvester pilot would unload the cuttings into the DNR boat using a second conveyor belt (Fig. 6).

Once the eelgrass cuttings were on board, biologists and volunteers sorted the material into mesh bags (Fig. 7). The bags were loaded onto a second vessel, which transported the filled bags back to Crisfield, MD. The bags were attached to lines at the dock and kept submerged in the ambient water overnight at Somers Cove marina. Each morning, the bags of harvested material were transported via commercial waterman to the DNR Piney Point Aquaculture facility in St. Mary's County, MD 24-48 hours after collection.

Figure 7 (above). DNR staff and volunteers load the seed material into bags.

Figure 8 (right). Bags of harvested material are loaded onto the transport boat bound for Piney Point.

Surveys done by DNR and VIMS after two years of harvesting concluded that the harvester did not have any significant impacts on the donor beds. Biologists swam through the areas harvested 2 weeks after harvesting, and could not distinguish between harvested and unharvested areas. Aerial surveys in 2005 over the areas harvested in 2004 clearly showed the cutting paths, but no decrease in acreage or plant density was visible.

Seed Processing

Once the bags of harvested eelgrass reproductive shoots arrived at Piney Point Aquaculture Facility, they were placed in one of eight, 20,000 gallon (32’x32’x4’) or one of sixteen 9,800 gallon (20’x20’x4’) greenhouse basins. The water in each basin was replaced daily with local St. Georges Creek water augmented with aquaculture grade sea salt to match conditions at the harvesting areas (~14ppt). In addition, each basin was aerated to prevent anoxia. Typical basin dissolved oxygen levels averaged 5-6 mg/l. Water quality was monitored twice daily in order to ensure adequate conditions. While in the basins, the eelgrass seeds slowly dropped from the reproductive shoots over the following month. After

Figure 9. Seed settling trays at Piney Point.

all the seeds were released and settled to the bottom of the basins, the seed/reproductive shoot slurry was pumped into a series of stacked settling trays to allow the passive accumulation of seeds while discarding the non-seed material.

Seed Storage

After the completion of seed processing, all seeds were placed in flow-through, aerated and salinity boosted holding tanks until fall seed dispersal. However, slight storage modifications were made each year in order to increase viable seed numbers. In 2004, the seed were stored in a series of three, 2000L cone shaped tanks. Because of the large volume of seeds and the concern for anoxic conditions in poorly mixed seeds, all tanks were heavily aerated to a "rolling boil." Eelgrass seed storage literature is extremely limited and the group consensus form DNR and VIMS was to try the higher levels of aeration. Extremely low numbers of viable seeds remaining by fall 2004 (~7%) required us to rethink storing seeds in the highly oxygenated system. In 2005, to more closely mimic successful storage conditions developed by VIMS, all seeds were held in a series of ten, 80L shallow tubs with lower aeration and frequent hand mixing to prevent accumulation of silt and possible anoxic conditions. Subsequently, a higher percentage of seeds remained viable for fall dispersal (~25%). While this was an improvement, levels were still below those regularly achieved at VIMS (>50%).

In order to address the issue of low numbers of seeds/ low seed viability, in 2005, a series of seed storage experiments were set up at Piney Point, VIMS (funded by Army Corps of Engineers) and St. Mary's College to determine optimum storage conditions. The following conditions were examined:

- Source of water
    - River water (filtered, unfiltered),
    - Re-circulated water (filtered, temperature controlled)
- Aeration level – High, low or no air
- Mixing – Seed mixing or no seed mixing
- Bleaching – Bleaching or no bleaching

Based on preliminary results, it appears that the most effective seed storage conditions involve using re-circulated water, with low aeration no mixing. However, we are in the process of evaluating all monitoring data and experimental seed storage methods to determine cause of the poor seed survivability so changes can be made to greatly improve seed viability in 2006.

Seeding Techniques

Seed Bags

A buoy-deployed seeding system (BuDSS) developed by Pickerell et al. was modified slightly and used as an alternative method to broadcasting bare seeds in the fall. There are several potential advantages to using this method, mainly pertaining to not needing to store seeds during the summer. For this method, reproductive material is placed in mesh bags immediately after harvest, moved to the restoration location, and deployed in the area to be restored. Immediate deployment of reproductive material eliminates the need to store seeds, reducing the number of seeds lost to processing and decreasing the expense and labor requirements associated with seed transport, processing, and storage.

As the bags of harvested material arrived at Piney Point, about 15,000 L were used to fill seed bags for deployment. DNR used a modified version of the buoy deployed seeding system, (BuDSS), created by Chris Pickerell at Cornell University Extension Service (Pickerell et al., 2003). Four gallons of collected reproductive shoots were placed in a mesh bag, divided into three sections by cable ties, and supported on each end with a small buoy. At one end, 2.1 m of polypropylene rope was attached to a cinderblock to anchor the seed bag (Fig. 10). The mesh bags suspended above the sediment allowing the seeds to mature and drop over a period of weeks, mimicking natural seeding events (Fig.11). Two types of seed bags were constructed and deployed: single (50,000 seeds) and double (100,000 seeds). Seed bags were deployed at the restoration sites by watermen and DNR staff for approximately one month (Fig.12).

The mesh bags remain suspended at the top of the water column, allowing the seeds to develop and drop over a period of weeks. This mimics the floating and rafting of reproductive shoots during natural seeding events during the natural phenological schedule (Pickerel et al. 2003). Although not proven, it has been suggested that this method may also reduce predation by spreading out seed dispersal over time and through a combination of time and natural forces yield a more even distribution of seeds.

There are potential problems with this method too. These include a navigational hazard while the mesh bags are on-site (restoration plots with floats every 10 meters are difficult to navigate). Despite staggering seed dispersal over time, seed predators are active during this time. Any sort of spring dispersal that mimics the natural dispersal will be affected by predators.

Figure 10. Picture of seed bag.

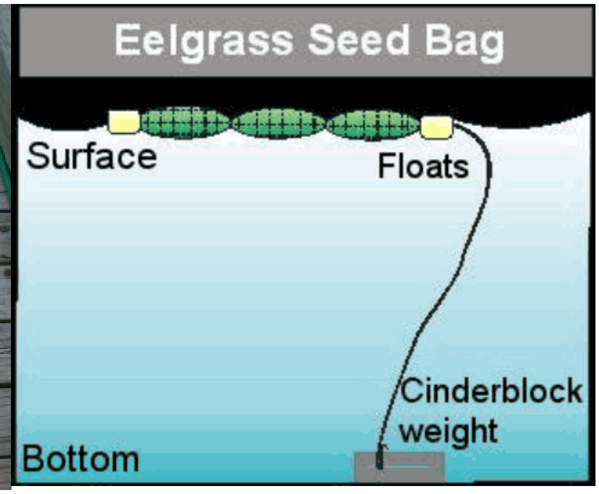


Figure 11. Schematic of seed bag in water column.

Figure 12. Seed bag deployment.

Seed Broadcast

This technique is effective because eelgrass seeds are rapidly incorporated into the sediment and generally do not move far for where they settle (Orth et al 1994). The complexity of the bottom due to biological and physical processes appears to be important to seed retention (Luckenbach and Orth, 1999). Eelgrass seed recruitment as a percentage of total seeds appears to always be quite low.

Annual seed production ranges from 6,176 seed m-2 to 24,460 seed m-2 (Olsen 1999) however, even during natural seeding, reported seedling numbers are significantly less than the numbers of seeds produced, ranging from 5-15 percent (Olsen and Sand- Jensen, 1994, Orth, 2003, Granger, 2002, Cook, 1979, Cabin et al., 2000). Researchers using seeds in experimental plantings have encountered varied success, but a common thread seems to be low germination rates (Moore et al, 1993), wash-out of seeds (Orth et al. 1994, Harwell and Orth 1999), and predation (Fishman and Orth, 1996).

Germination in the Chesapeake Bay is thought to be dependent upon temperature, burial, and oxygen cues (Orth and Moore, 1983, Moore 1993). Incorporation of seeds into the sediments (Orth and Moore, 1983, Moore 1993) is essential for the initial of germination. Microtopography prevents long distance redistribution of seeds (Orth et al 1994, Luckenbach and Orth, 1999). Orth et. al. (1994) demonstrated that turbation of the sediment as little as 1 millimeter deep could stop an eelgrass seed from rolling and being transported away. However, deep burial can stop seed germination. Deep burial of seeds below the redox potential discontinuity prevents the developing plant from receiving light (Bigeley, 1981), which may be crucial to germination.

Although not made before the seedings took place, observations by divers during the 2005 surveys at each of the sites suggest that the bottom at each site on the Potomac was suitable for seed recruitment. Seed predation also appears to be an important factor in seed loss (Janzen, 1971, Wassenberg, 1990 and Fishman and Orth, 1996). Experiments where predation was eliminated yielded 100% germination rates illustrating the importance of seed predation (Fishman and Orth, 1996). One mechanism employed by plants to escape predation is to produce seed abundances high enough to satiate the seed predator (Orth, 2003).

Due to the physical presence of three-dimensional structure provided by SAV, and the increased "roughness" of the bottom in SAV beds, water velocities are reduced as much as 50% reduced within SAV beds (Fonseca et. al 1982,, Benoy and Kalff 1999, Gacia et. al 1999). Furthermore, it has been noted that water velocity reductions are directly proportional (as a power function) to both the height and the growth form of the species that occur in the area (Gacia et. al 1999, Petticrew and Kalff 1992).

Eelgrass seeds were hand broadcast using methods used by Orth (Orth, Personal Communication) during the fall of 2003. The restoration site was divided into seven 25 m radius plots 1963.4 m^2, or 0.485 acres. The plots were divided into 5m concentric circles from a central point. The concentric areas at 5m increments were chosen to evenly allocate the seeds across the plot by broadcasting while walking around the plot in concentric circles (Fig. 13). To distribute at a density of 100,000 seeds/acre, 50,000 seeds or 660 ml, were broadcast with the appropriate proportions going to each concentric section. For example, 237mL of seeds (36% of the total 660mL) went into the outer ring (green).

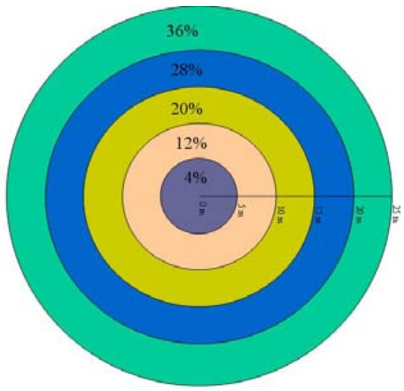


Figure 13. Diagram of the methodology used to disperse seeds by hand using concentric rings (percentages indicate portion of the total seeds distributed in each ring).

This method was slow and did not guarantee an even distribution of seeds. Subsequent seed broadcasts in the fall 2004 were achieved mechanically using a specially designed seed broadcast apparatus developed by C & K Lord and Associates and DNR staff. All seed broadcasts took place before ambient water temperatures dropped to 15^{0}C, the temperature at which eelgrass seeds begin germination. In Maryland, seeds were mechanically dispersed using a newly developed seed-sprayer from C& K Lord, Inc capable of evenly dispersing seeds at suitable densities (200,000 seeds/acre) at the rate of 10 minutes/acre (Fig. 14).

Restoration efforts with eelgrass in plots of different sizes (4 m^2 to 400m^2) and configurations (alternating 4 m^2 patches and large continuous patches) in different river systems in Virginia (VIMS) have shown a significant site but no significant plot size effect (Orth, personal communication). To look at seeding density effects, Orth et al. (2003) tested five seeding densities ranging from approximately 10,000 seeds/acre to 5,000,000 seeds/acre and found no density dependent effects on germination rate or seedling success. The effectiveness of seeding density was also tested in this project in order to evaluate the potential for site-specific variation in density dependence. Nominal seeding density treatments of 10,000, 50,000, 100,000, and 500,000 seeds/acre were tested. Treatment densities were assigned to randomly chosen plots within the restoration site. The number of treatments, replicates per treatment, and size of plots was dependent upon the number of seeds available. The number of treatments and plot size was reduced as necessary in order to maintain sufficient replication for statistical rigor. After spring surveys (May 2006) the effectiveness of seeding density will be closely examined to evaluate the potential for site-specific variation in density dependence.

Figure 14. Seed sprayer

Eelgrass spring seed bag and fall seeding sites were located adjacent to State Highway Administration (SHA) sites planted with adult plants at the Piney Point site. Seeds were hand broadcast during the fall of 2003, and by boat in 2004, and 2005. Seeds were broadcast before ambient water temperatures dropped to 15^{0}C, the temperature at which eelgrass seeds begin germination. In Virginia, Orth et al. (2003) tested five seeding densities ranging from approximately 10,000 seeds/acre to 5,000,000 seeds/acre and found no density dependent effects on germination rate or seedling success. The effectiveness of seeding density was also tested in this project in order to evaluate the potential for site specific variation in density dependence. Nominal seeding density treatments of 10,000, 50,000, 100,000, and 500,000 seeds/acre were tested. Treatment densities were assigned to randomly chosen plots within the restoration site. The number of treatments, replicates per treatment and size of plots was dependent upon the number of seeds available. The number of treatments and plot size was reduced as necessary in order to maintain sufficient replication for statistical rigor.

Water Quality Monitoring

The Strategy calls not only for large-scale SAV restoration projects, but also for coincident assessment of the associated habitat conditions in order to evaluate reasons for success or failure and, in turn, improve the likelihood of success of future projects. DNR conducts temporally and spatially intensive monitoring in Maryland's tidal waters to fully characterize ambient water quality conditions in open and shallow waters. These data have been employed to assess EPA water quality criteria such as dissolved oxygen, chlorophyll, and water clarity, as well as characterize habitat conditions for bay grasses and aquatic organisms. DNR, in association with the Chesapeake Bay Program, has developed consistent monitoring and analysis protocols for these monitoring programs.

Continuous Monitoring

Each continuous monitoring station is equipped with a YSI 6600 water quality monitoring sonde. Beginning in 2004, all YSI 6600 data sondes are equipped with Extended Deployment Systems (EDS). The EDS has a wiper system that allows the continuous monitoring sondes to be deployed for longer periods of time without suffering a degradation of data quality as a result of biofouling. Each continuous monitoring sonde records nine water quality parameters every 15 minutes. The nine water quality parameters measured continuously are water temperature, specific conductance, salinity, dissolved oxygen, turbidity (NTU), fluorescence and total chlorophyll (used to estimate chlorophyll *a*), pH and water depth.

Continuous monitoring sondes are positioned in the water column in either a floating configuration that suspends the sonde at some distance below the surface (usually 1 meter), or in an anchored configuration that fixes the sonde at some distance above the bottom. The sonde position is determined based on the geographic area being monitored and the monitoring goals for that segment. Continuous monitoring sondes in a floating configuration are suspended from a float inside of a 4-inch diameter PVC pipe with 2-inch holes drilled every 4 inches below the waterline to allow for water exchange. Sondes in a fixed configuration are also housed inside a perforated 4-inch diameter PVC pipe, and a bolt is used to hold the negatively buoyant sonde at a fixed depth above the sediment bottom.

Several times a day, the computer server located at the Bay Program office contacts the data logger located at each sampling site via TCP/IP communications and then uploads, archives, and updates the data display on the Eyes on the Bay web site. These data are available immediately on the Internet, allowing the general public to view near real-time water quality data. Details of the steps for installing, calibrating, deploying, and retrieving the YSI instruments are fully provided in DNR's Quality Assurance Project Plan.

In addition to the parameters measured by the sonde, Secchi depth and light attenuation are measured weekly from April to October, and grab samples are taken and filtered on-site or immediately after returning to the laboratory. The processed samples are sent to the Nutrient Analytical Services Laboratory (NASL) at the Chesapeake Biological Laboratory and to the Maryland Department of Health and Mental Hygiene (DHMH) for analysis. These results are used to analyze relationships between the water quality parameters measured by each continuous monitor and the nutrient component. Some of the lab data were also used to check the YSI data for accuracy. Parameters analyzed at NASL are total dissolved nitrogen, particulate nitrogen, nitrite, nitrite + nitrate, ammonium, total dissolved phosphorus, particulate phosphorus, orthophosphate, dissolved organic carbon, particulate carbon, silicic acid, total suspended solids, volatile suspended solids, particulate inorganic phosphorus and dissolved organic carbon. Parameters analyzed at DHMH include chlorophyll *a*, pheophytin and turbidity.

Water Quality Mapping

Water quality mapping is conducted using water quality mapping, a shipboard system of geospatial equipment and water quality probes that measure water quality parameters from a flow-through stream of water collected near the water's surface. Water quality mapping measured are water temperature, salinity, conductivity, dissolved oxygen, turbidity (NTU), fluorescence (used to estimate chlorophyll *a*) and pH. The water is pumped through a ram (pipe), through the sensors, and then discharged overboard. The water quality mapping unit includes a hand-held Garmin global positioning system (GPS), a microcomputer, and a YSI 6600 sonde with a flow-through chamber. Each water quality datum collected is associated with a date, time, water depth, and GPS coordinate (WGS84).

Water quality mapping allows data to be collected rapidly (approximately every four seconds) while the boat is traveling at speeds of up to 25 knots. The water quality mapping system is compact and can fit onto a small boat, allowing sampling in shallow water and the ability to map an entire small tributary such as the Severn River in less than a day. The distance between samples depends on vessel speed; generally at least one observation is collected approximately every 30 meters (100 feet). The water quality mapping system samples water at approximate 0.5-m depths below the surface. Real-time data are displayed in the field through custom software, either in numerical and graphical form or in a real-time mapping application, DATAVIEW, developed by MD DNR.

At 5 to 8 calibration stations per tributary segment, grab samples are collected at 0.5-m depth and filtered on site. The processed samples are sent to the Chesapeake Biological Laboratory and to the DHMH for analysis. Parameters analyzed at NASL total dissolved nitrogen, particulate nitrogen, nitrite, nitrite + nitrate, ammonium, total dissolved phosphorus, particulate phosphorus, orthophosphate, dissolved organic carbon, particulate carbon, silicic acid, total suspended solids, volatile suspended solids, particulate inorganic phosphorus and dissolved organic carbon.

Parameters analyzed at DHMH include chlorophyll *a*, pheophytin and turbidity. In addition, Secchi depth and photosynthetically active radiation (PAR) measurements are taken at calibration stations to calculate light attenuation (Kd).

The calibration station locations are selected to: 1) sample the greatest possible range of water quality conditions found during each cruise; 2) sample a broad spatial area; 3) overlap with long-term fixed monitoring and continuous monitoring stations.

Monitoring Seedling and Vegetative Shoot Success

Surveying and Monitoring

Germination rates, seedling survival, and growth in each seeding density replicate were assessed annually at approximately 1 month, 6 months and 12 months after seeding following methods similar to that of Orth et al. (2003). However, since DNR seeded larger areas than described by Orth et al. (2003), seedling density of seed plots were subsampled by counting the total number of seedlings along diagonal transects between the four corners of the planting area. The areas outside of the original plots were also surveyed to make sure that the broadcast seeds remained within the plots. The number of plants was estimated visually using methods similar to that of Orth et al. (1999). Finally, to determine whether the created eelgrass beds are expanding through vegetative propagation and/or natural seeding, the seed plots and surrounding area were surveyed in the spring and fall following each seeding using aerial overflights and groundtruthing with a handheld mapping GPS.

Test plantings were carried out to ensure that areas identified by the site selection model would support growth of eelgrass. Adult eelgrass plants were transplanted into 3 - 1 m^2 test plots located adjacent to seed broadcast or seed bag areas. A density of 64 adult plants per m^2 was used for each test plot. These test plots were monitored at the same time and frequency as the seed plots.

Results:

Due to the differences in seeding methods and results at each site, the results are presented by individual site with a summary analysis at the end. The methodology for seed dispersal, seed bags deployment, and monitoring are the same for each site. However, due to differences in bathymetry, currents, and obstructions, the size of each area will not be uniform.

Seed collection

2003: Reproductive shoots from healthy eelgrass beds containing mature seeds were collected manually in Tangier Sound. Harvesting took place on May 20, 23, and 27-30 and yielded 2.3 million seeds, 250,000 of which were viable for broadcast.

2004: A mechanical harvest boat was utilized to increase the efficiency and amount of reproductive material collected. From May 24 to June 4, 2004, seeds were collected from donor beds in the Little Annemessex River. In nine cutting days the mechanical harvester collected approximately 71.92 L of eelgrass reproductive material. In 2004, the portion of reproductive material transported to Piney Point for seed extraction yielded 15.12 million seeds. After the seed processing and storage process was complete, 7% of the collected seeds (or 1,058,400 seeds) were viable for broadcast.

2005: Reproductive material was harvested from the Little Annemessex River and the mouth of Acre Creek (Big Annemessex River) from May 23 to June 8, 2005 (Fig. 5). The harvest machine collected approximately 109.5 L of eelgrass seeds from 21.6 acres of eelgrass beds. Seed count estimates were made after all of the seeds had fallen from the reproductive shoots and were separated from the decaying reproductive material. Replicate 2 ml samples of seed material were analyzed for the number of viable seeds. The total number of seeds harvested was calculated as the sum of the number of seeds per ml (113/ml) and the total volume of seeds collected (109.5 L). Based on this calculation, the portion of reproductive material transported to Piney Point for seed extraction yielded 12,373,500 seeds. An estimate of the number of viable seeds was also determined as the sum of the number of viable seeds (68 viable seeds/ml) and the total volume. Using this calculation, there were an estimated 7,446,000 viable seeds, 60 percent of the total number of seeds collected, after processing was through. After storage of the seeds throughout the summer, there were a total of 2,527,000 viable seeds.

In spring 2003, 2.3 million seeds were gathered by hand using snorkeling and SCUBA equipment with a majority of the seeds coming from Sinepuxent Bay. From that harvest, 250, 000 seeds were available for harvest, giving a yield of 11%. The seeds were taken to Piney Point, where they were separated and maintained in storage containers for the fall 2003 hand dispersal. The seed material harvested in this fashion contained more seeds per bag because the divers/snorkelers could differentiate the reproductive shoots. While the seeds counts per bag were considerably higher than later years, this method required more than 500 man hours and was extremely time consuming, so improvements were made in subsequent harvests.
Whether for use in fall seed broadcasts or spring seed bags, it is necessary to know the number of viable seeds in order to achieve predetermined seeding densities and to determine the subsequent recruitment rate (number of seedlings/number of viable seeds distributed). For the spring seed bag method, the number of seeds placed into seed bags was estimated by counting seeds in four 1L subsamples of reproductive material and multiplying the resulting seeds/L by the total volume of harvested material. This gives us an estimate of the total number of seeds dispersed using the seed bag method. However,

because we never extract the seeds from the spathes to analyze each of them individually there is no direct measure of the number of viable seeds vs. dead or non-viable seeds. Therefore, recruitment is the number of seedlings recruited/the total number of seeds distributed.

Seeds to be used for fall seed broadcast are separated from reproductive material at the Piney Point facility. Two methods were used to count seeds to be used for fall seed broadcasts, one before the seeds separated from reproductive material, and one after seeds had been processed. In 2005, the portion of reproductive material transported to Piney Point for seed extraction yielded between 12 and 32 million seeds. The 32,806,200 seed estimate was determined shortly after collection by counting seeds in four 1L replicate subsamples of reproductive material and multiplying the resulting seeds/L (210 seeds/L) by the total volume of harvested material (149,800 L). This gives us an estimate of the number of seeds dispersed using the seed bag method because we never extract the seeds from the spathes to count them directly. The 12,373,500 seed estimate was made after all of the seeds had fallen from the reproductive shoots and were separated from the decaying reproductive material.

The total number of seeds at Piney Point in 2005 was calculated using the number of seeds per ml (113/ml) compared to the total volume of seeds collected (109.5 L). An estimate of the number of viable seeds was also determined using replicate 2 ml samples of seed material. The number of viable seeds (determined using the squeeze test) per mL was compared to the total seed volume. Using this calculation, the 68 viable seeds/ml can be extrapolated to predict 7,446,000 million total viable seeds.

The first seed count method estimates the totally number of seeds collected. However, because this method does not account for seed losses through any number of processes it may not accurately reflect the true number of seeds available for broadcasting. While the other method estimates number of seeds and determines the viability of seeds, it too has some sources of uncertainty. Because good seeds separate from bad seeds in water, it is necessary to drain all of the water from the seed slurry and completely mix the seed mixture before obtaining a representative sample. In addition, human error is a factor in both measuring samples out as well as the squeeze test for viability. When measuring aliquots, seeds are very sensitive to packing, creating a lot of variability in total seed number between the 2 ml samples. During the squeeze test a seed is deemed viable or not viable based on physical robustness of the seed. There is considerable subjectivity in this determination as well. Efforts were made to keep the methods as uniform as possible, but because of the vast number of counts that are made it is not feasible to use the same staff member to conduct all counts. We have not been able to determine to what degree these sources of error affect our estimates and thus can't determine the best estimate.

Seed Dispersal and Test Plantings

Seeds were dispersed by hand in 2003. Seven rings of 5m each were seeded with 50,000 seeds per ring (Fig. 3). This site was adjacent to the Woodrow Wilson Bridge Mitigation project, allowing for a side by side comparison of the effectiveness of planting seeds vs. adult shoots.

In 2004 and 2005, seeds were dispersed in the spring and fall. In 2004, 2,400,000 seeds were dispersed in the spring and 262,500 in the fall. In 2005, 4,510,000 seeds were dispersed in the spring and 400,000 seeds were dispersed in the fall. Listed below is a summary of the seeds dispersed, the method of dispersal, along with field observations made during the monitoring for each site

Piney Point

DNR biologists used the ring method developed by Orth (Personal Communication) to disperse the 250,000 viable seeds available at the time of dispersal in 2003. Adjacent to the 2003 hand broadcast areas, 150,000 seeds were broadcast in a 0.5 acre plot in fall 2004 (Fig. 3).

Table 1. Piney Point Seeding Results

Site	Seeding Method	Sampling Date (2005)	Plot Size (Acres)	Number of seeds	Plants per acre	Estimated plants in plot
Piney Point	Seed Broadcast	05/12/2005	0.5	150,000	0	0
		8/1/2005			0	0
		11/3/2005			0	0

St. George Island

In spring 2004, seed bags containing 605,000 seeds were dispersed in a 5 acre plot (Fig. 3). The site was monitored for the first time on May 12, 2005 and there were 567 eelgrass plants observed per acre, with an estimated 2,835 eelgrass plants in the entire plot.

In the fall of 2004, 75,000 seeds were dispersed by machine broadcast in a 0.3 acre plot. The fall seeding area had 586 eelgrass plants per acre on May 12, 2005 for an estimated 147 plants in the plot.

Test plantings placed at each site in November 2004 were monitored on the same dates. In May 2005, an average of 55 plants were observed among the three test plots at St. George Island, yielding an 86% initial planting success rate. In August, 6% of the plants remained, half of which survived through November 2005.

Table 2. St. George Island Seeding Results

Site	Seeding Method	Sampling Date (2005)	Plot Size (Acres)	Number of seeds	Plants per acre	Estimated plants in plot
St. George Island	Seed bags	05/12/2005	5	605,000	567	2835
		08/1/2005			369	1985
		11/3/2005			45	213
	Machine Broadcast	05/12/2005	0.25	75,000	586	147
		08/1/2005			1246	312
		11/3/2005			37	9

Sage Point

In 2004, there was only spring seed bag dispersal at this site. There were two sites, each with 605,000 seeds spread over 5 acre plots (Fig. 3). Field observations made by biologists identified large amounts of widgeon grass, snails, and live oysters on the bottom.

Table 3. Sage Point Seeding Results

Site	Seeding Method	Sampling Date (2005)	Plot Size (Acres)	Number of seeds	Plants per acre	Estimated plants in plot
Sage Point	Seed bags	05/12/2005	5	605,000	509	2545
		08/1/2005			0	0
		11/3/2005			0	0
	Seed bags	05/12/2005	5	605,000	128	641
		08/1/2005			0	0
		11/3/2005			0	0

Test plantings placed at this site in November 2004 were monitored on the same dates. In May 2005, an average of 52 plants was observed among the three test plots at Sage Point, yielding an 81% initial planting success rate. In August and November of 2005, no plants were observed.

Cherryfield Point

In 2004, there was a spring seed bag and fall seed broadcast at this site. In the spring, two adjacent 2.5 acre plots were seeded with seed bags with 275,000 seeds dispersed in each plot (550,000 total, Fig. 3).

Table 4. Cherryfield Point Seeding Results

Site	Seeding Method	Sampling Date (2005)	Plot Size (Acres)	Number of seeds	Plants per acre	Estimated plants in plot
Cherryfield Point	Seed bags	05/12/2005	2.5	275,000	437	1092
		08/1/2005			16	39
		11/3/2005			0	0
	Seed bags	05/12/2005	2.5	275,000	32	50
		08/1/2005			0	0
		11/3/2005			0	0
	Machine Broadcast	05/12/2005	0.25	37,500	0	0
		08/1/2005			0	0
		11/3/2005			0	0

Test plantings placed at this site in November 2004 were monitored on the same dates. In May 2005, an average of 11 plants was observed among the three test plots at Cherryfield Point, yielding a 17% initial planting success rate. In August and November, no plants were observed.

Water quality

The SAV strategy calls not only for large scale SAV restoration projects, but also for assessment of the associated habitat conditions in order to evaluate reason for success or failure and to improve the likelihood of success for future projects. In keeping with the requirement of this strategy, long term, fixed and continuous water quality monitoring was conducted for 2004 and 2005. Data from the continuous monitoring stations and the water quality mapping cruises were analyzed to explain the seed germination and plant survival results. In addition, the 2004 data will be compared to the nearby Potomac River Mainstem cruise 20 year data record to assess if conditions during this project period were anomalous.

Light availability and temperature are the two most critical water quality parameters for Z. marina (Stankelis, 2003). In the Chesapeake Bay, there is a well-documented bimodal eelgrass growth pattern with primary growing season beginning when temperatures rise above 10oC with a peak in biomass occurring in late May to early June (Orth, review). A second, less dramatic growing season occurs in mid-September and continues until water temperatures drop below 10oC sometime in November. Increasing light attenuation and water temperature (above 25oC) later in June cause decreased growth and leaf defoliation (Moore et al. 1996; 1997).

The continuous monitoring data provide an in depth record for some of the parameters (turbidity, temperature) that affect SAV during the summer season. Four graphs that summarize data collected for 2004 and 2005 at the Piney Point and Sage Point water quality monitoring stations (Fig. 15 & 16).

Water quality mapping and Potomac River Mainstem cruises are marked on the graphs (Fig. 17-19). The red line indicates a Nephelometric Turbidity Unit (NTU) of 5.38, the turbidity compensation depth, (the water depth above which plants at 1M deep will not receive the light necessary to carry out basic metabolic functions). On the 2005 graphs, dates when DNR monitored the plants at each site were also marked. The Potomac River experienced turbidity values above 5.38 for most of the summer. The actual values on a particular day are not as important as the number of consecutive days these values were above the turbidity compensation depth.

Piney Point

Data was recorded at the Piney Point Water Quality monitor from March through the end of October 2004. In 2004, the turbidity values exceeded the 5.38 NTU maximum for 18% of the year and exceeded the maximum for 18% of the SAV growing season. In 2004, the records indicate that temperature did not exceed 30^0C for the entire data record. In 2005, the values exceeded the 5.38 maximum for 7% of the year and also for 7% of the SAV growing season. In 2005, the records for temperature data show that temperature exceeded this 30^0C for 2.6% of the year.

Sage Point

In 2004, the Sage Point continuous monitor showed that the turbidity exceeded the 5.38 maximum 27% of the year, and exceeded that value for 26% of the SAV growing season. In 2004, the records indicate that temperature did not exceed 30^0C for the entire data record. Turbidity was lower in 2005, and only exceeds the 5.38 NTU limit for 17% of the year. Turbidity during the SAV growing season also exceeded the limit 17% of the time. In 2005, the records for temperature data show that temperature exceeded this 30^0C for 2.6% of the year at Sage Point.

Fluorescence was another parameter monitored by the continuous monitor stations. Correlation values were determined for turbidity from the 2004 and 2005 data sets. At the Piney Point Site, the Pearson Correlation Coefficient was 0.08175 (P Value= .0001, N = 17707) in 2004 and 2005 yielded a 0.07070 Pearson Correlation Coefficient (P Value= .0001, N = 18441). At Sage Point in 2004, the data yielded a -0.01153 Pearson Correlation Coefficient (P Value = 0.1072, N = 19524). The data for 2005 yielded a 0.14798 Pearson Correlation Coefficient (P Value =.0001, N = 16674).

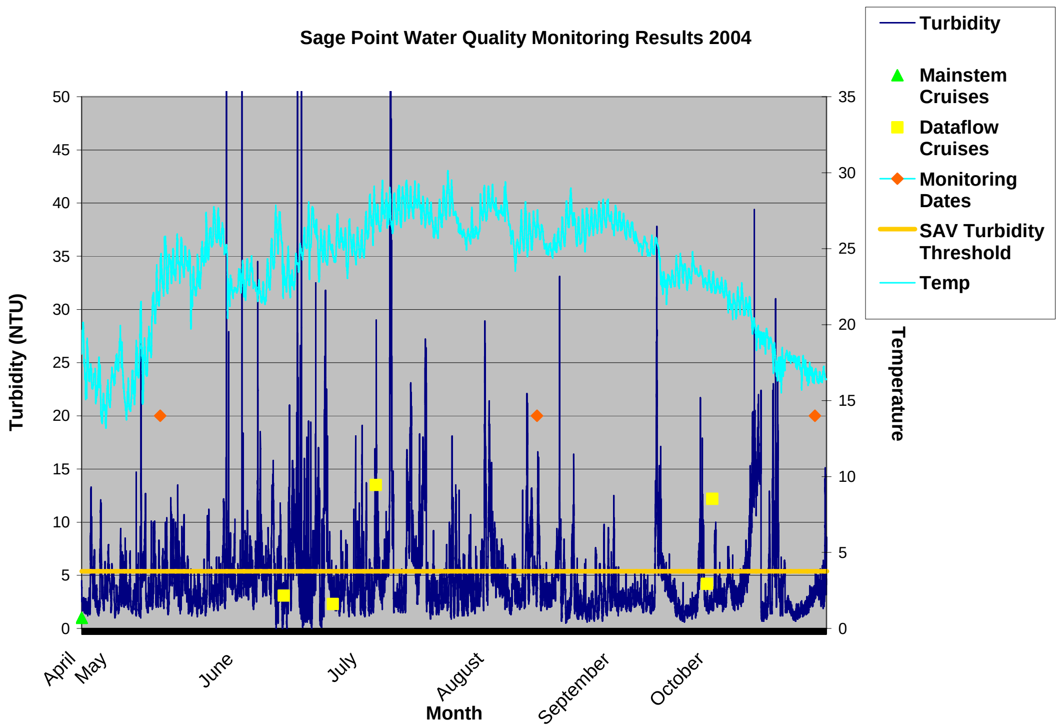
Water Quality Mapping

Water quality mapping data are also represented as a map due to the large number of data points. Cruise pattern data are interpolated to provide graphs indicating parameters levels. Each graph shows a picture of the turbidity conditions present in the Potomac for each month. The data show a picture of the water quality for the whole river at a fixed point in time, and can be used in conjunction with continuous monitoring data to identify small scale differences.

Water quality mapping was conducted monthly throughout the eelgrass growing season (March-November) throughout the lower portion of the river. Turbidity data were compiled for 2004 and 2005. Water quality mapping cruises were conducted and turbidity data were analyzed from April to October 2004 and April to October 2005, with 2 cruises in each month in 2005. In 2004, turbidity peaked in June, with values between 5-7.5 NTU's. In August and September 2004, there was a spike in turbidity upriver from the St. George Island site and there were patches of high turbidity around the restoration site. In 2005, there was a similar spike in turbidity in June, August and September at St. George Island. The rest of the sites remained unaffected, with values lower than 2.5 for most of the year.

Finally, data from the Mainstem bay monitoring cruises collected Secchi depth from 2003-2005. Due to the large amount of data points, these data are represented graphically in relation to the 20 year average. The graphs compare the Secchi depth readings collected for each year, compare it to the mean for the prior years, and show the range of data over the 20 year period.

Figure 15. Continuous monitoring data for Sage Point, 2004 and 2005.



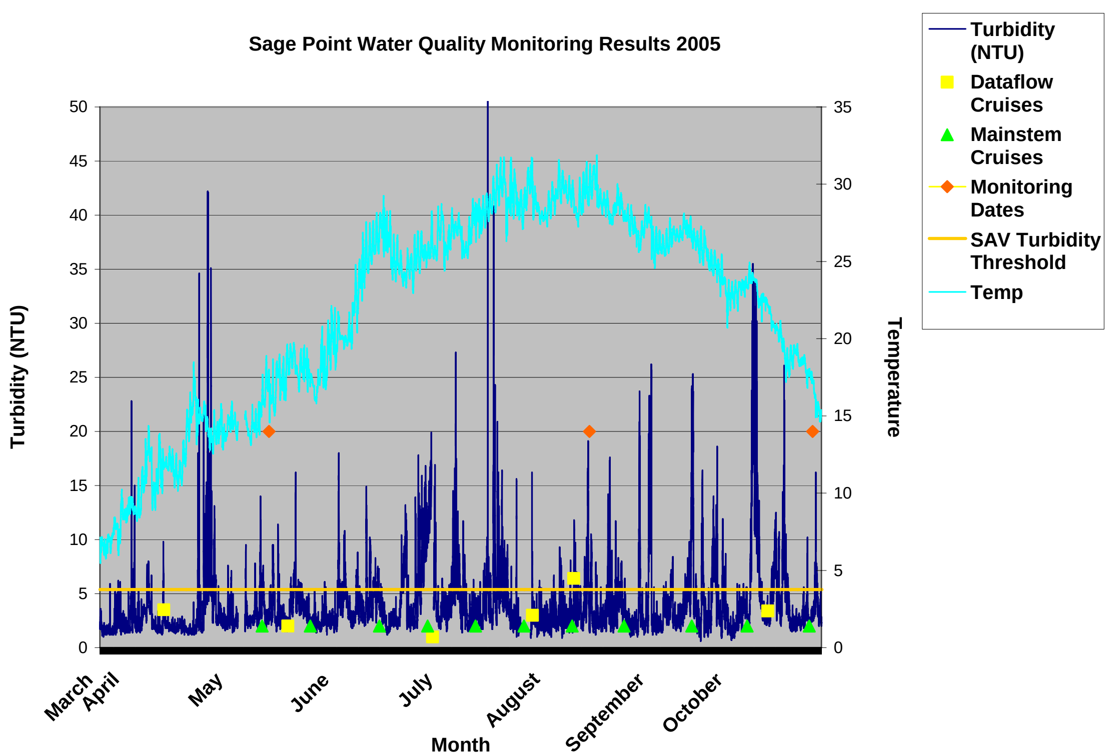
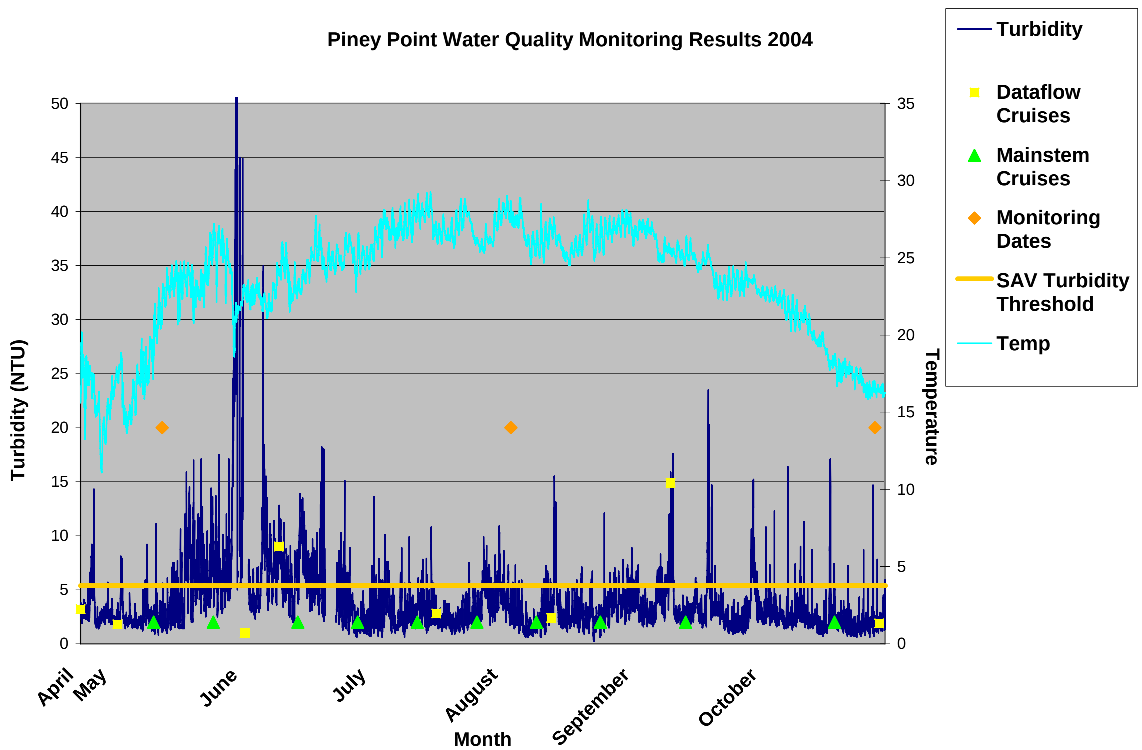


Figure 16. Continuous monitoring data for Piney Point, 2004 and 2005.



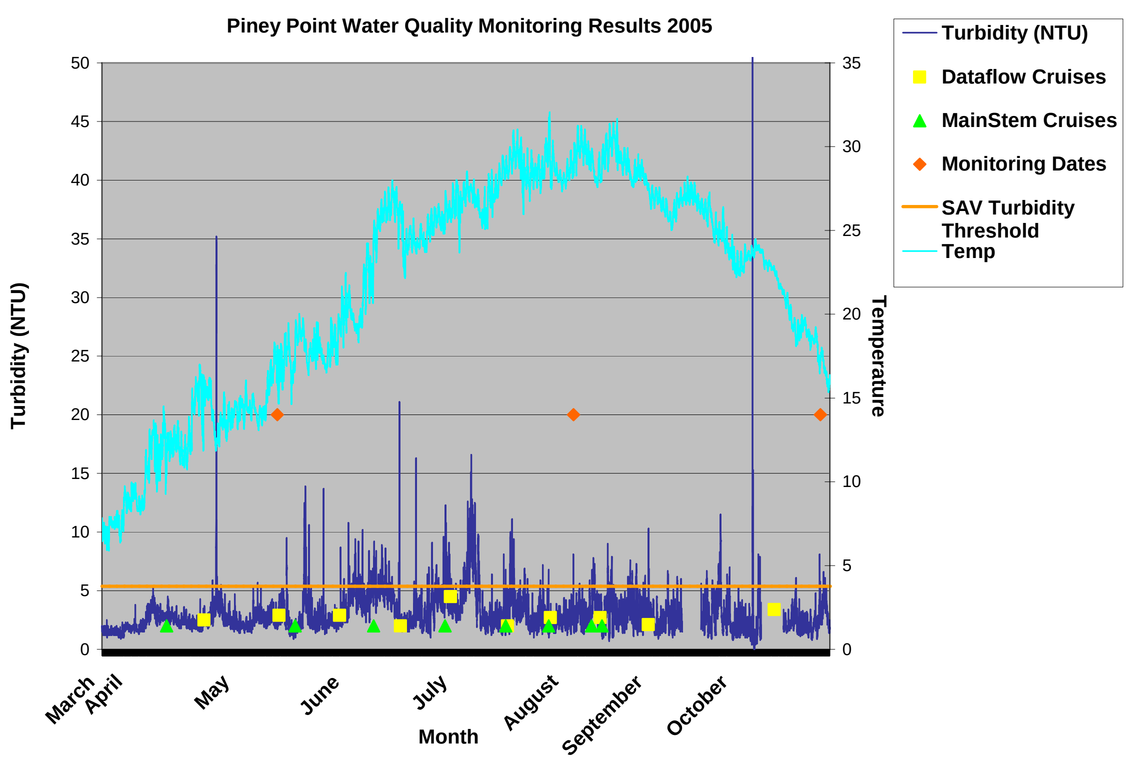


Figure 17. Turbidity data from 2004 Water Quality Monitoring Cruises.

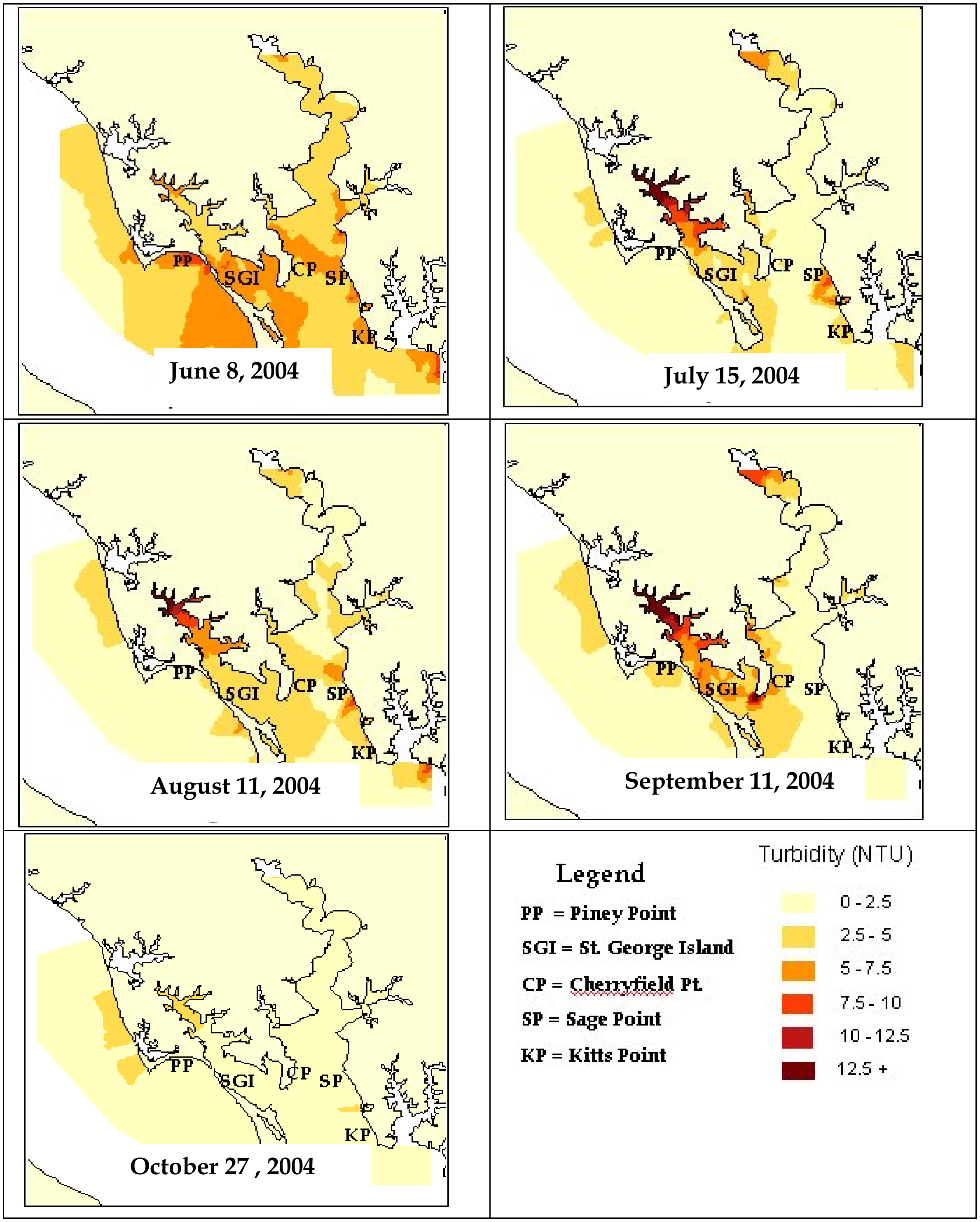


Figure 18. Turbidity data from water quality mapping cruises, 2005.

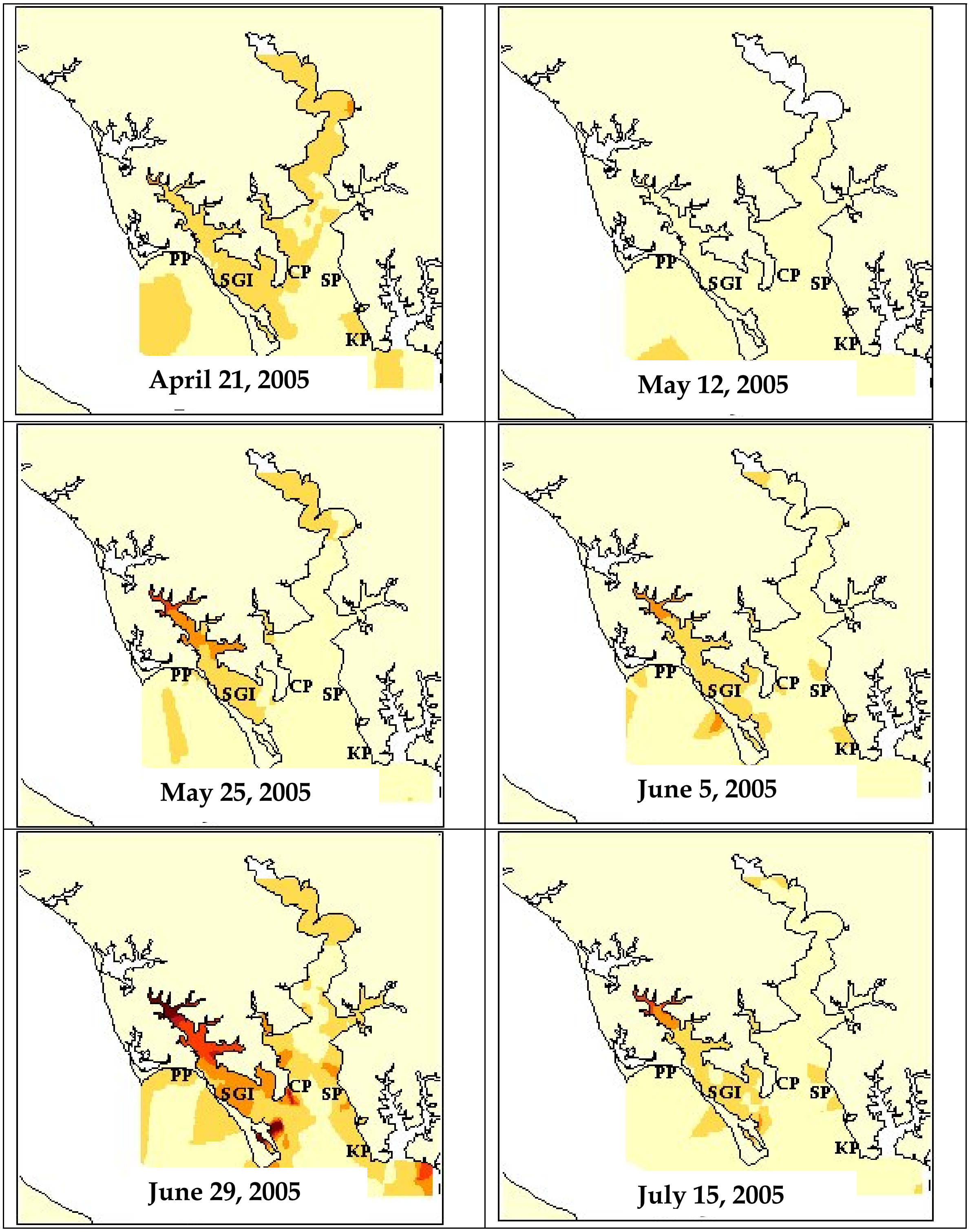


Figure 18 (cont'd). Turbidity data from 2005 water quality mapping cruises

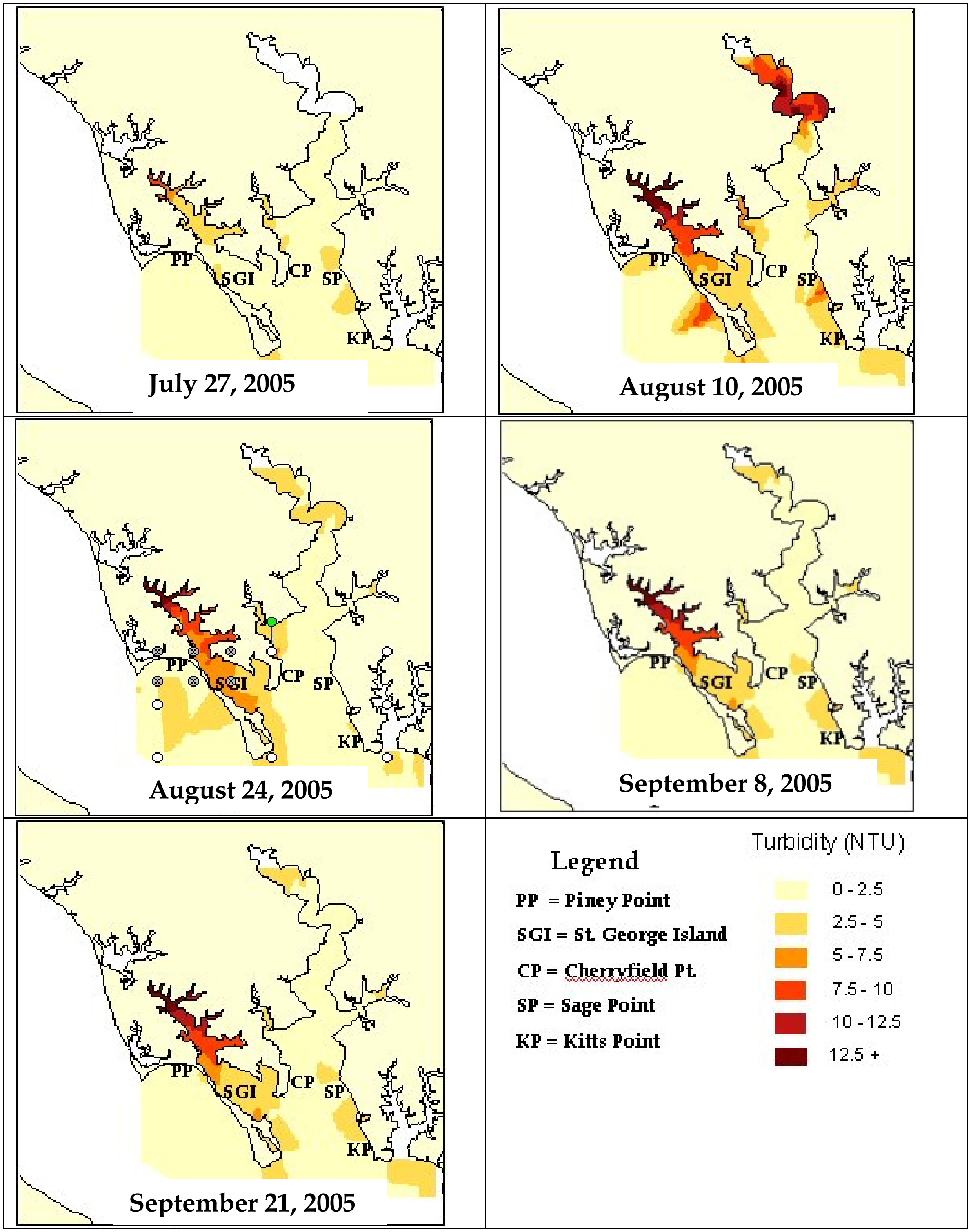


Figure 19. 2003-2005 Secchi Depth at Point Lookout Monitoring Station

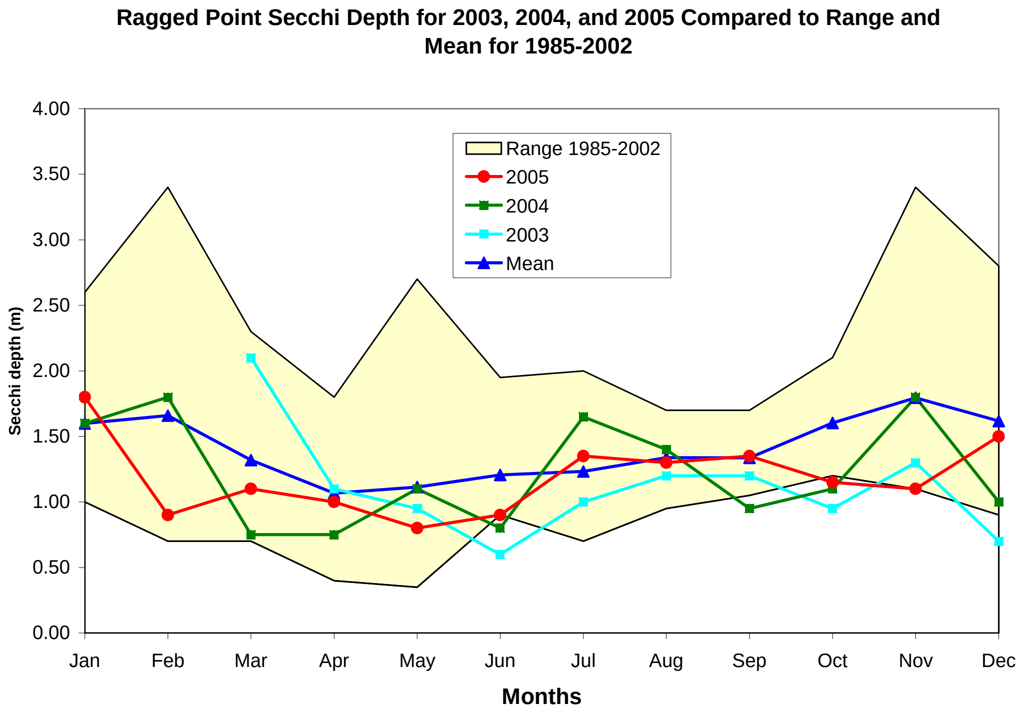


Table 5. Water quality mapping data for 2004, Sage Point and Piney Point

Site	water quality mapping CRUISE	Turbidity Value(NTU's)		Site	water quality mapping CRUISE	Turbidity Value(NTU's)
Piney Pt.	May	2.5-5		**Sage Pt.**	May	2.5-5
	June	7.5-10			June	5-7.5
	July	0-2.5			July	5-7.5
	August	0-2.5			August	2.5-5
	September	2.5-5			September	0-2.5

Table 6. water quality mapping Cruise Data for 2005, Sage Point and Piney Point

Site	water quality mapping CRUISE	Turbidity Value(NTU's)		Site	water quality mapping CRUISE	Turbidity Value(NTU's)
Piney Pt.	May	0-2.5		**Sage Pt.**	May	0-2.5
	June	2.5-5			June	2.5-5
	July	0-2.5			July	0-2.5
	August	2.5-5			August	0-2.5
	September	0-2.5			September	0-2.5

Mitigation Plantings

Survival was monitored at one month, six months and twelve months, and was estimated as a percentage of the original planting that survived. Of the 2003 plantings, at one month the sago pondweed had a 10.4% survival rate, widgeon grass had a 6.9% survival rate, and eelgrass had a 33.7% survival rate. At 6 months, eelgrass had a 26.7% survival rate, but the other two species did not survive. After 12 months, 9% of the eelgrass survived.

For 2004, at one month 3% of the sago pondweed, 9.1% of the widgeon grass, and 50.2% of the eelgrass survived. At 6 months, 3% of the sago pondweed, 9% of the widgeon grass, and 37.9% of the eelgrass survived. After 12 months none of the plants remained.

At the Sage Point site in 2004, at one month sago pondweed had a 10.1% survival rate and widgeon grass had a 0.1% survival rate. After 6 months no plants survived.

Seeding method cost comparison

In the spring of 2004, 20 acres were covered with seed bags, with approximately 2.4 million seeds distributed. In the fall, 1 acre was seeded by machine broadcast, distributing 262,000 seeds. The estimated number of plants for the spring seeding was 7,193, and the estimate for the fall was 147.

The total cost for seeding one acre was calculated by multiplying the cost per seed by the specified seeding density (200,000 seeds/acre). The recruitment success of each method was determined by dividing the total number of seeds dispersed by the number of successfully recruited plants. The total cost for each method was divided by the total number of successfully recruited seedlings to determine a ratio of cost per successfully recruited seedling between the spring seed bag and fall seed dispersal methods.

The cost per seed put out in Maryland for 2004 was $0.02 for the spring seed bag method and $0.34 for the fall seed broadcast. The total cost for seeding one acre was determined by multiplying the cost per seed by the specified seeding density (200,000 seeds/acre). The cost for restoring one acre was determined to be $4,473 for the spring seed bag method and $67,085 for the fall seed broadcast method.

The spring seed bag method yielded 7,193 seedlings across all spring seed bag sites locations out of 2.4 million seeds broadcast, a recruitment success rate of 0.3%. The fall seed broadcast method yielded 147 seedlings across all fall seed broadcasts locations out of 262,000 seeds were dispersed, a recruitment success rate of 0.06%.

Each seedling (7,193) successfully recruited using the spring seed bag method cost $1.70. Each seedling (147) successfully recruited using the fall seed broadcast method is $363.89. For the purpose of cost comparison between methods, site selection and monitoring costs were not included. At the Piney Point site, RK&K engineers planted 15,000 eelgrass PUs, 1,600 widgeon grass PUs and 946 PUs of sago pondweed plants. Their cost per plant was $4.70.

Discussion

Early eelgrass restoration efforts in the Chesapeake Bay involved transplanting adult eelgrass plants from healthy source beds to restoration locations. Averaging $37,000 per acre not including monitoring (Fonseca, 1998), this and other restoration methods are both expensive and labor intensive and can damage donor beds. Despite some advantages to using adult plants (e.g. successful adult plants yield reproductive shoots during the following year's reproductive season, Orth, 2003), seed broadcasting appears to be a more efficient and cost effective restoration technique with the added benefit of having less impact on donor beds (Orth, 2000).

The results of the 2003-2004 restoration efforts have highlighted some of the obstacles to large-scale seed broadcasting. Seed storage represents the largest investment of time and resources, yet due to high seed mortality in the summer, relatively few viable seeds have been broadcast in the fall. Water quality plays a critical role in the survival of seedlings that have germinated. The results from each site vary greatly, so the discussion will be divided by site.

Site Selection

The most important step of any restoration project is selecting the proper location. Many previous restoration projects have suffered from improper siting (Harrison 1987; Fonseca 1992). The sites used for eelgrass restoration for this project were chosen using the DNR targeting system, which is designed to assess large areas of the Bay for their restoration potential. The model uses six layers of data to evaluate the suitability, ability and potential of a particular habitat to support SAV populations. After the targeting system identified areas, the sites selected underwent a two-year site selection process of test plantings and water quality monitoring. However, through test plantings and evaluation of 2003 and 2004 results, the Piney Point and Sage Point sites were found to exhibit less than ideal conditions. In additions, some factors that are difficult to include in the targeting system, like future water quality conditions, current velocity, and sediment type can have negative effects on seed establishment. Unfortunately, it is only through test plots and intensive monitoring that these factors can be evaluated. In 2006, DNR will increase seeding efforts in the sites that were most successful in 2004 and 2005.

Seed Collection

Two methods were used to count seeds, one for the spring seed bag method and one for fall seed broadcast method. The number of seeds dispersed using the spring seed bag method was determined shortly after collection by counting the total number seeds in four, 1 L replicate subsamples of reproductive material and multiplying the resulting seeds/L by the volume of seed material in each seed bag, and then the number of seed bags in a given plot.

The seed estimate for the fall seed broadcast method was made after all of the seeds had fallen from the reproductive shoots and were separated from the decaying reproductive material.

The first seed count method (used for spring seed bags) estimates the total number of seeds collected and included in each seed bag. As not all the seeds in every spathe can be expected to be viable, it may not accurately reflect the true number of viable seeds dispersed. The method used to enumerate seeds for the fall seed broadcasts also determines the viability of seeds. As such, it is likely to be more accurate. However, this method also has some sources of uncertainty. Because good seeds separate from bad seeds in water, it is necessary to drain all of the water from the seed slurry and completely mix the seed mixture before obtaining a representative sample. In addition, human error is a factor in both measuring samples out as well as the squeeze test for viability. When measuring aliquots, seeds are very sensitive to packing, creating a lot of variability between the 2 ml samples. During the squeeze test a seed is deemed viable or not viable based on physical robustness of the seed. There is considerable subjectivity in this determination as well. Efforts were made to keep the methods as uniform as possible, but because of the vast number of counts that are made it is not feasible to use the same staff member to conduct all counts. We have not been able to determine to what degree these sources of error affect our estimates.

In 2003, eelgrass seed harvesting was done using SCUBA and snorkeling gear to harvest reproductive shoots by hand. While this method was effective, the labor involved in these collections was very great, and only 2.3 million seeds were collected- ultimately limiting the size of potential restoration areas. In 2004, the use of a mechanical harvester allowed for the collection of 15.12 million seeds with roughly the same effort as 2003. In 2005, additional staff and boats further increased the efficiency of mechanical seed collection.

When using the mechanical harvester to collect eelgrass reproductive shoots, a number of steps were taken to minimize impact to the donor beds. As the reproductive shoots extend above the vegetative shoots, the depth of the cutting blades on the harvester were kept high enough above the sediment that the rhizome mat and lower parts of the eelgrass plants were not disturbed. As a further precaution, harvesting took place over a large area to assure that sufficient seeds remain for bed maintenance (Granger, 2002). To confirm that there was no significant damage to eelgrass beds where reproductive shoots had been harvested, divers used SCUBA to survey the harvested beds 8 weeks (July 22, 2004) after the 2004 collection. Divers reported abundant, healthy eelgrass and quite a bit of flowering widgeon grass. There were no substantial differences in plant height, bed density, or apparent vigor of the plants themselves between the harvested and unharvested beds. In addition, aerial photography taken on June 19 and July 6, 2004 confirmed that the areas that were harvested in May were still densely vegetated (VIMS; 2004 field observations and aerial photography accessible: [http://www.vims.edu/bio/sav/2004 obs.html#vims071304](http://www.vims.edu/bio/sav/2004obs.html#vims071304)).

Test plantings

Test plots for each of the sites were planted in November of 2004, and surveyed in spring 2005. The test plots for the Sage Point had an 81% initial survival rate, but lost all of the above ground biomass before our August sampling. Cherryfield Point yielded a 31% initial survival rate, and also lost all biomass before August. St. George Island had an 86% initial survival rate, but unlike Sage Point and Cherryfield Point, 3% of the plants remained at the site in November. For 2006 efforts, a much higher proportion of seeds will be placed at the St. Georges Island location to attempt to build on this success.

The Piney Point seeding and seed bag site was located adjacent to the RKK Engineers eelgrass restoration site that was planted with adult plants. This allows for a side-by-side comparison of the adult plants and seedling. Of the eelgrass planted in 2003, after 6 months, 27% of eelgrass survived, and at 12 months less than 10% of all adult eelgrass plants remained.

Seed Viability

Storing the spring-harvested seeds through the summer is one of the most difficult aspects of this project. Each year there has been a substantial loss of seeds during seed storage, ultimately decreasing the number of viable seeds at the end of the storage process and reducing the acreage of SAV restored. Two million three hundred thousand seeds were collected in 2003, of the half that were stored at Piney Point through the summer, only 250,000 of these were viable and used for fall broadcast. Harvest efforts in 2004 collected 15.12 million seeds. However, only 7% of these (1,058,400 seeds) were deemed viable in the fall. The 2005 harvest collected 12.4 million seeds total. After distributing about half of the seeds in spring seed bags, 109.5 liters containing approximately 7,446,000 seeds remained at Piney Point. Unfortunately, only 2,527,000 seeds were viable at the end of the seed processing/storage procedure. After the 2004 season, biologists from VIMS and DNR attempted to identify potential problems with the seed transport and separation and holding/storage process. The lack of basic research on seed physiology made identifying specific problems very difficult. In 2005, seed storage experiments were set up at St. Mary's College, VIMS, and MD-DNR to test the impact of the following parameters: flow, aeration, salinity, and stirring. When the results of these experiments are analyzed, appropriate modifications will be made to the seeds processing and storage procedure to be applied to the 2006 seed collection.

Seed Bags/Seed Dispersal

In May, 2004, seed bags were deployed across 20 acres, with approximately 2.4 million seeds distributed. In the fall, 262,000 seeds were machine broadcast in a 1 acre plot. The estimated number of plants resulting from the spring seeding was 7,193, and the estimate for the fall was 147. Two factors could have contributed to the difference in the results; variance in number of seeds dispersed, and the time of year the seeds were dispersed. Assuming the seeds were the same, the difference in the number of seeds dispersed could explain the difference in recruitment. Regardless of the reason for the variance observed, spring seeding clearly was the most effective method for seed distribution in 2004.

Except for Piney Point, where no plants were observed, all of these sites had much greater success with the spring seed bag dispersal. Since the same seed bag material was used for Piney Point, this site appears to not have the restoration potential of the others, and will not be used in 2006. For fall seed broadcast, the recruitment was much lower due to poor seed survival during storage.

The results for the number of plants generated for each method was one of the most important components of this experiment. Data from this project will be used by DNR and other organizations to guide future large-scale restoration efforts. In looking at the raw numbers, it states a very clear case for using seed bags for future restoration. Both cost per seed distributed and cost per recruited eelgrass shoot was less expensive than fall broadcasting.

These results were compiled using all available data from the 2005 plants resulting from 2004 seeding efforts. One of the major factors in the lower costs was the lack of viable seeds the end of the summer. Seed storage has been one of the more difficult obstacles in this project. Each year, refinements have been made to increase seed viability, but the percentage of viable seeds per seed collected is still very low. DNR has been working with VIMS to make further improvements to this phase of the project. DNR will make modifications to the seed storage in 2006 to minimize the loss of viable seeds.

Eelgrass Survival related to water quality

Eelgrass seed distributions in 2004 resulted in the successful establishment of seedlings at each site in May, 2005. Clearly, seeding areas of the Potomac River could be an effective method for initiating eelgrass growth. However, almost all adult test plot plants and seedlings completely disappeared in the summer of 2005. If conditions were ideal and the plants had simply undergone summer defoliation, we would have expected to see those plants again during the November survey, during their fall growing period. This was not the case. Very few plants were seen during the November survey, which suggests that the plants died rather than underwent a seasonal defoliation.

To determine the cause for the near complete loss of adult plants, water quality data from the continuous monitoring stations, mainstem stations, and the water quality mapping cruises were analyzed to detect trends or spikes in water temperature and turbidity data that may explain these results. A number of studies have shown that decreased light availability affects eelgrass survival (Philips et al. 1978; Kemp et al. 1983; Dennison and Albert 1986; Twilley et al. 1985). Eelgrass requires between 6 and 8 hours of photosynthetic saturating irradiance per day to survive (Dennison and Alberte, 1985). Although it is not well documented how many days healthy plants can survive elevated turbidity and decreased light availability, it is not likely that the recruited seedlings or adult plants could survive the prolonged periods of high turbidity such as those reflected by the continuous monitor data. When water clarity data for 2003, 2004 and 2005 are compared to the 20-year record, the values are below the mean each year, with 2003 being the year with the worst water clarity (Fig. 19).

Turbidity values are one measure we have to determine light availability in the Potomac during our study period. Using the EPA requirement of 22% of surface irradiance for healthy SAV growth, and an application depth of 1.0 meter, a turbidity value of 5.38 NTU's was determined as the water clarity target for the mesohaline portion of the Potomac River.

The data reported here reflect the results of 2004 seeding efforts. Germination of these seeds took place in the fall of 2004, and those seedlings were subject to fall 2004 water quality upon germination. Keeping in mind the importance of light, when we look closely at the turbidity conditions occurring between our three sets of surveys, it is evident that there were episodes of severely elevated turbidity (turbidity higher than the 5.38 NTU threshold) at both of the continuous monitoring stations. At the Piney Point Station, turbidity levels were above the threshold for 34 days, with a majority of those days between May 1 and June 15, 2005. The water quality mapping data show that turbidity levels exceeded the threshold for the June and July cruises at each site. For the Sage Point site, the turbidity levels exceeded the threshold for 26% of the year, all of which fell during the growing season. The water quality mapping data also show that turbidity levels exceeded the threshold in the months of June and July. The 2005 results showed that turbidity exceeded for 7% of the growing season at Piney Point, and 17% at Sage Point, but the water quality mapping data show that turbidity levels were below the threshold at each site for the entire summer.

Total Suspended Solids (TSS) and chlorophyll (calculated from fluorescence) concentrations are the two primary contributors to turbidity throughout Chesapeake Bay. In order to determine which parameter contributed most to light attenuation in the Potomac, correlation values were determined between turbidity and chlorophyll from the 2004 and 2005 continuous monitoring datasets. At the Piney Point station in 2004 and 2005, the Pearson correlation coefficients were 0.08 ($P < 0.0001$, $N = 16972$) and 0.005 ($P < 0.0001$, $N = 18441$, respectively). At the Sage Point station, 2004 and 2005 had Pearson correlation coefficients of 0.02 ($P < 0.0001$, $N = 17447$), and 0.0001 ($P < 0.0001$, $N = 19524$, respectively). These regression analyses indicate a weak correlation between chlorophyll and turbidity at both stations for both years. This weak correlation suggests that suspended solids are the primary contributor to the high turbidity in the Potomac River.

Temperature is the other water quality parameter that directly affects eelgrass survival. Eelgrass in the Chesapeake Bay is near the southernmost extent of its distribution on the east coast of the United States. Eelgrass growth slows and defoliation occurs at temperatures above 25^0 C (Moore et al 1996 and 1997). Compounding this effect, when temperatures increase above just 20^0 C, epiphyte loading increases substantially (Dr. Walter Boynton, personal communication). To look at potential temperature effects during this study, a maximum threshold for eelgrass survival of 30^0 C was adopted. A comparison of the Piney Point and Sage Point sites shows that the temperature data were nearly identical in 2004, with both stations showing the temperatures below 30^0 C for the entire season. In 2005, the Piney Point site recorded a temperature above 30^0 C for 2.6% of the season and the Sage Point site for 3.4% of the season (Fig. 15 & 16). Although it is not well documented how many days healthy plants can tolerate these elevated temperatures, the fact that these instances of elevated turbidity coincide with elevated temperatures are the likely reason that most recruited seedlings and adult plants did not survive the summer of 2005.

In surveys done by VIMS at the end of the 2005 summer, there was a widespread defoliation of eelgrass beds in the Virginia portion of the Chesapeake Bay. Eelgrass typically undergoes an annual, summer defoliation, with detached leaves forming large floating wracks that end up on beaches around the bay (Orth, personal communication). This normally takes place in late June or early July. In 2005, however, the die-off was much more severe, with several areas that had been vegetated for many years suffering a complete loss of above-ground biomass. In Virginia, the eelgrass loss is suspected to be due to a combination of higher than normal summer water temperatures, low winds, and lower than normal light levels (Orth, personal communication).

Cost Comparison

The large seed loss during storage (80%) is responsible for the significantly higher costs per seed and per acre using the fall seed broadcast method. If 50% of the total seeds retained throughout the processing and storage procedure, a total of 1,871,000 viable seeds would have been available for broadcast on the Potomac River. With the additional viable seeds and same total costs, the cost per seed would be reduced from $0.34 to $.04 and the cost per acre would drop from $67,085 to $7,157. This is a reasonable expectation, since VIMS retained 80% of total seeds as viable in 2005 (Orth, personal communication).

The high costs of processing associated with the seed broadcast method combined with the seed losses during storage make it significantly more expensive than dispersing seeds using the spring seed bag method. In order for fall seed broadcasting to achieve the same seed cost as the seed bag method ($0.02/seed and $4,473/acre) 2,992,000 seeds would have to be broadcast on the Potomac of the total 15.12 million seeds collected. In order for this to occur, seed viability would need to be increased significantly and a larger proportion of the total viable seeds would have to be allocated for the Potomac River than in previous years.

The recruitment success of each method was determined by dividing the total number of seeds dispersed by the number of successfully recruited plants. However, there was considerable difficulty in determining the number of viable seeds, so this analysis is largely speculative. The spring seed bag method yielded 7,193 seedling spread across all spring seed bag locations. A total of 2,400,000 seeds were dispersed using this method. Therefore, the recruitment success for the seed bag process was 0.30%. The fall seed broadcast method yielded 147 seedlings, requiring 262,000 seeds to achieve a 0.06% recruitment rate.

The total cost for each method was divided by the total number of successfully recruited seedlings to determine cost per successfully recruited seedling. Each seedling (7,193) successfully recruited using the spring seed bag method cost $1.70. Each seedling for the fall seed dispersal method is $363.89. At this point in the study, spring seed bags are a much more cost effective restoration technique than fall seeding. However, other restoration efforts using the fall seed broadcast method in Maryland (DNR) and Virginia (VIMS) resulted in recruitment rates ranging from .5 to 14% in 1999 and 4.3 to 13.8 in 2000. If DNR recruitment rate improved to be similar those observed at VIMS, the cost per successfully recruited plant would be $40.83-$1.46 according to the 1999 rates and \$4.75-\$1.48 according to 2004 rates. This would bring the two techniques much closer in terms of cost effectiveness.

A spring seed bag project conducted in 2004 in VA (VIMS) resulted in a recruitment rate of 1.3%. Initial restoration efforts using the BuDSS in the Peconic Estuary, NY yielded 7% recruitment (Pickerell, 2003). If DNR recruitment rates for the seed bag method ranged from 1.3-4.0%, the cost per recruited plant would drop to \$0.39-\$0.13. The projected cost of \$0.39 per plant at a 1.3% recruitment rate would still be more cost efficient than the cost per plant using the seed broadcast with a 14% recruitment rate. Regardless of which method is used, it is clear that we must work to improve recruitment rates if this project is to have the potential for truly large-scale restoration.

Mitigation Plantings

The final report from RKK engineers indicated some possible reasons for the failure of the adult shoots. In some of the planting unit grid locations no plant material could be found, but planting anchors were located. This would indicate that some of the plant losses were due to poor installation. In other instances, root and rhizome material along with dead leaves were located anchored by the skewer. These rhizomes themselves were found to be dead and decaying. These observations indicate that some failure was due to plants not surviving the process of packing, shipping, holding or installation. For the 6 month survey, about half of the plants that were counted as not surviving appeared to have been dead prior to planting, and half appeared to be the result of poor installation.

Observations of the sago pondweed grids indicated similar reasons for failure. Half of the non-surviving plants appeared to have died after installation while the other half appeared to have been installed incorrectly. Of course, the major difference between sago and eelgrass plots is that almost all of the sago pondweed installed were counted as non-surviving. Widgeon grass PUs were counted as non-surviving with less frequency than Sago, however the reasons appeared to be roughly the same. Since DNR was not involved in the planting process, it is difficult to quantify if these plants failed for the reasons above, or the losses were primarily due to unfavorable water quality conditions.

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